ABSTRACT

Title of Thesis: FACTORS AFFECTING MERCURY CONCENTRATIONS IN 3 SPECIES OF STREAM SALAMANDER IN GARRETT COUNTY, MARYLAND

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I sampled 9 streams in Western Maryland during the spring, summer and fall of 2010. 5 species of stream salamander were collected at these streams and analyzed for total mercury; northern dusky salamanders (*Desmognathus fuscus*), northern two-lined salamanders (*Eurycea bislineata*), Allegheny Mountain dusky salamanders (*Desmognathus ochrophaeus*), seal salamanders (*Desmognathus monticola*), and eastern red spotted newts (*Notopthalmus viridescens*). The streams were also analyzed for various water chemistry factors; pH, Acid Neutralizing Capacity (ANC), Total Mercury (THg), Methyl Mercury (MeHg), Chloride (Cl^-), Nitrate (NO_3^-), Sulfate (SO_4^{2-}), Total Suspended Solids (TSS), and Dissolved Organic Carbon (DOC).

In all but two streams adult northern two-lined salamanders had significantly higher concentrations of total mercury in their tissues than larval northern two-lined salamanders. Adult northern two lined salamanders also had the highest concentrations

of the three species that were statistically analyzed; northern two-lined salamanders, northern dusky salamanders, and Allegheny Mountain dusky salamanders. Stream methyl mercury concentrations and stream DOC were also found to significantly influence salamander tissue total mercury concentrations.

FACTORS AFFECTING MERCURY CONCENTRATIONS IN 3 SPECIES OF STREAM SALAMANDER IN GARRETT COUNTY, MARYLAND

By

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INTRODUCTION

Mercury is a toxic pollutant of global interest. Mercury can remain in the atmosphere for as long as two years in its elemental form. The primary anthropogenic source of mercury to the atmosphere is coal fired power plants. Once mercury enters the atmosphere it can be deposited to soils and other surfaces, eventually making its way to wetlands and waterways. Once in the wetlands and anoxic sediments, anaerobic bacteria can methylate the mercury. Methyl mercury can then bioaccumulate eventually being biomagnified in higher trophic levels. Mercury concentrations in fish and other top predators have received significant research, but we know little about mercury concentrations in other organisms such as salamanders. Salamanders are uniquely connected to both the aquatic environment and surrounding riparian areas. The connection allows them to act as vector to transport mercury from aquatic food webs into terrestrial food webs that would otherwise receive little exposure to mercury.

The focus of my research was on the concentrations of mercury found in five species of salamander from nine streams in Garrett County, MD. We collected individual northern two-lined (*Eurycea bislineata bislineata*), northern dusky (*Desmognathus fuscus fuscus*), Allegheny Mountain dusky (*Desmognathus ochrophaeus*), and seal salamanders (*Desmognathus monticola*) as well as eastern red-spotted newts (*Notopthalmus viridescens viridescens*). I also concurrently collected water samples from each of my streams in order to determine what if any influence water chemistry factors may have on salamander total mercury concentrations. There have been few studies on the mercury concentrations found in salamanders, but those studies do indicate significant concentrations can be found in salamanders from both streams with low mercury concentrations and contaminated streams with high concentrations (Bank et al. 2005, Bergeron et al. 2010, Burke et al. 2010).

The lack of thorough knowledge of the mercury concentrations in salamanders led me to develop three main objectives for my study:

1. Determine the pattern of mercury concentrations in the different life stages of stream salamanders.
2. Determine the patterns of mercury concentrations across different species of stream salamander.
3. Determine the patterns of mercury across different streams and determine any influence water chemistry has on that pattern.

These three objectives are addressed separately in three chapters, which will be submitted for publication. Chapter 1 focuses on mercury concentrations measured in adult and larval northern two-lined salamanders. Northern two-lined salamanders were the only species where larvae were collected in sufficient number to allow comparison with adults. Chapter 2 focuses on comparing mercury concentrations in adult northern two-lined, northern dusky, and Allegheny Mountain dusky salamanders. These three species were collected from six streams to allow for comparisons across different watersheds. Chapter 3 focuses on water quality factors across all nine streams and how they are statistically related to total mercury concentrations in salamanders.

Three appendices are included to address data that was collected but not included in the above chapters. Mercury concentrations measured in eastern red-spotted newt individuals and seal salamander individuals are included in appendix A. Appendix B includes methyl mercury concentrations measured in a small subset of salamanders used to determine the percent methyl mercury in salamander tissues. This data was not included in the chapters because the analysis did not meet quality control standards. Appendix C includes salamander community data collected from all nine streams. This includes the total number of salamanders encountered at each stream, the total man-hours spent searching for salamanders, and the Shannon Diversity Index calculated from the salamander presence data.

CHAPTER 1: VARIATIONS IN TOTAL MERCURY CONCENTRATIONS BETWEEN ADULT AND LARVAL NORTHERN TWO-LINED SALAMANDERS IN STREAMS OF GARRETT COUNTY, MARYLAND

ABSTRACT

The purpose of this study was to increase our understanding of mercury bioaccumulation in northern two-lined salamanders (*Eurycea bislineata bislineata*). We measured mercury concentrations in adult and larval northern two-lined salamanders, which were collected from seven streams in Garrett County, Maryland in April, July and September of 2010. Averaged over all streams, adult northern two-lined salamanders had significantly higher concentrations of total mercury than larval salamanders (29.5 ng g^{-1} vs. 22.1 ng g^{-1}). Across all streams, the methyl mercury concentrations in stream water were positively correlated with the total mercury concentrations in adults and larvae. Dissolved organic carbon (DOC) in these streams was also positively correlated with total mercury concentrations in larvae. Total mercury concentrations in adult northern two-lined salamanders were significantly greater than total mercury concentrations in larval northern two-lined salamanders at five of seven streams: Bear Creek, Bear Pen, Mill Run, Monroe Run, and Poplar Lick. In contrast, total mercury concentrations in adult and larval northern two-lined salamanders were not significantly different at Mud Lick and Little Savage River. In addition, salamanders from these two streams had the highest total mercury concentrations compared to all other streams. The lack of difference between adult and larval salamander total mercury concentrations in Mud Lick

and Little Savage River suggests that dietary pool of mercury available to the larvae plays an important role in the bioaccumulation of mercury in this species.

INTRODUCTION

Mercury is a neurotoxin that is released into the environment through processes such as metal manufacturing, waste incineration, mining, and most commonly, from coal-fired power plants used to generate electricity. This airborne mercury enters terrestrial and aquatic ecosystems primarily from atmospheric deposition. Moore et al. (2011) estimated total atmospheric mercury deposition rates of 30 $\mu g\ m^{-2}\ y^{-1}$ for ecosystems in western Maryland (Moore et al. 2011). After mercury is deposited to aquatic ecosystems, the inorganic mercury can be converted into methyl mercury (CH_3Hg) by anaerobic bacteria (Gilmour et al. 1991; Morel et al. 1998). This methyl mercury can subsequently be bioaccumulated in the tissues of aquatic organisms where it acts as an endocrine disruptor and/or immune suppressor.

Mercury dynamics in stream ecosystems have been extensively studied in the eastern United States. Total mercury concentrations ranged from 0.09 ng L^{-1} to 80 ng L^{-1} and methyl mercury concentrations ranged from <0.04 ng L^{-1} to 3.2 ng L^{-1} (Shanley et al. 2005). Previous studies in Garrett County, Maryland reported total mercury concentrations in streams ranging from 0.42 ng L^{-1} to 6.76 ng L^{-1} with an average concentration of 1.38 ng L^{-1} (Castro et al. 2007). Methyl mercury concentrations in these same streams ranged from 0.04 ng L^{-1} to 0.55 ng L^{-1} (Castro et al. 2007). Mason et al. (2000) reported average total mercury concentrations of 1.7 ng L^{-1} and 2.1 ng L^{-1} in two other streams in western Maryland. The mean methyl mercury concentrations in these streams were 0.01 ng L^{-1} and 0.06 ng L^{-1}. Total mercury concentrations in western Maryland streams exceed concentrations in streams in Shenandoah National Park (0.24 to

0.65 ng L^{-1}) by factors of 2 to 10 and are similar to average concentrations (1.7 ng L^{-1}) in streams in Acadia National Park (Moore et al. 2009; Peckenham et al. 2007).

These streams are home to many species of salamanders, which are sensitive to environmental pollutants dissolved in stream waters (Lannoo 2005; Petranka 1998). Mutations in many species of salamanders have been linked to environmental pollutants such as atrazine, an herbicide, and nitrate fertilizer, which can be absorbed through the skin of adults and through the permeable mucosa surrounding their eggs (Boone and James 2003; Hatch and Blaustein 2003; Lenkowski et al. 2008; Rouse et al. 1999; Storrs and Kiesecker 2004; Taylor et al. 2005; Williams et al. 2008). However, the impact of mercury on stream salamanders is not well understood. There have been only three studies of mercury and stream salamanders in the eastern United States (Bank et al. 2005; Bergeron et al. 2010; Burke et al. 2010). The unique physiology of salamanders and their complex life cycles may make them more effective indicators of ecosystem mercury contamination than other stream dwelling organisms, such as fish and insects (Bank et al. 2005; Southerland 2004). Greater understanding of mercury dynamics in salamanders will provide new insight into the movement of mercury through the environment and processes responsible for the bioaccumulation of mercury in salamanders.

This study focused on mercury concentrations in both adult and larval northern two-lined salamanders (*Eurycea bislineata bislineata*) across several small streams in Garrett County, Maryland. Our objectives were to document patterns of total mercury concentrations in northern two-lined salamanders in different life stages and to identify differences in salamander mercury concentrations amongst different streams. We hypothesized that adult northern two-lined salamanders would have lower mercury concentrations than larvae. This hypothesis assumed that the mercury concentrations in the terrestrial diets of the adult salamanders are significantly lower than the mercury concentrations in the aquatic diets of the larval salamanders. In addition, we hypothesized that salamanders from streams with higher mercury concentrations would have higher total mercury concentrations.

METHODS

Study Streams

We studied salamanders in seven first-order streams in western Maryland. Five streams were located in the Savage River watershed and two streams were located in the Youghiogheny River watershed (Fig. 1). Mean pH and ANC in these streams ranged from 5.98 to 6.70 and from 43.9 μeq L^{-1} to 467.8 μeq L^{-1} respectively. These small streams drained watersheds dominated by forest (62% to 97%) and agriculture (<1% to 29%). Watershed area ranged from 95 ha at Bear Creek to 4,365 ha at Mill Run. However most watersheds were less than 2,200 ha (Table 2). Our study streams and watersheds are similar to those found throughout the Appalachian Plateau region of western Maryland.

Field Sampling

We collected northern two-lined salamanders in April, July, and September of 2010 using visual encounter surveys. To collect adult salamanders, cover objects were overturned within 2 m of the stream edge. To collect larval salamanders, cover objects were overturned within the first 0.5 m of the stream channel. Larval salamanders were identified by the presence of external gills. Survey sampling for salamanders lasted for 2 to 6 hours at each stream. Adults and larvae were collected using a small aquarium dip net. After capture, they were transported in glass jars to the Appalachian Laboratory (AL) in Frostburg, MD. We attempted to collect 10 adults and 10 larvae per stream for each sampling. The total number of samples at individual streams ranged from 2 to 30 for adults, and 5 to 30 for larvae (Table 1). Dry conditions during summer likely reduced the number of individuals collected. High flow events just prior to sampling also reduced the number of larvae encountered. This may be due to high flow conditions forcing larval salamanders downstream of the sampling site. Two egg masses were removed from gravid females collected at Monroe Run and Mill Run for total mercury analysis.

Water Chemistry

Stream water was sampled monthly from April through December 2010 with the exception September. Measurements of water temperature, specific conductivity, and pH were made *in situ* using a Hydrolab Quanta model sonde. Grab samples of stream water were collected in acid cleaned high density polyethylene (HDPE) bottles for lab analysis of closed pH, ANC, total and methyl mercury, dissolved organic carbon (DOC), total suspended solids (TSS), chloride (Cl^{-1}), nitrate (NO_3^{-1}), and sulfate (SO_4^{-2}). Water samples were filtered through 0.45 µm Whatman glass fiber filters for TSS and DOC. Mercury samples were collected in ultra clean 1 L Teflon bottles and double Ziploc bagged using the "clean hand/dirty hand" technique (EPA method 1639). All samples were placed on ice and transported in coolers to the AL for analysis. Samples were stored at -4ºC and were analyzed within 1 month of collection using EPA protocols (Table 3).

Total Mercury in Salamanders

At AL, the live salamanders were euthanized in a buffered 10 g L^{-1} solution of methane tricainesulfonate (MS-222). Once euthanized, salamanders were measured for weight, total length, and snout-vent length (SVL). Salamanders were then double bagged and frozen at -22ºC until analyzed. All analyses were completed within 3 months of sample collection. Prior to analysis, salamanders were thawed and allowed to reach room temperature. Afterwards, whole salamanders were digested overnight in an acid solution of 70% concentrated sulfuric acid and 30% concentrated nitric acid. The following day, 100-150 µL of digestion solution and 250 µL of bromine mono-chloride (BrCl) were added to 50 mL of distilled de-ionized water in separate 65 mL ultra-clean FEP Teflon auto-analyzer vials. The total mercury concentration in this solution was measured using cold vapor atomic fluorescence spectroscopy (CVAFS) in a Tekran 2600 CVAFS mercury analysis system in the class 100 clean room at AL (EPA Method 1631).

Quality Assurance/Quality Control (QA/QC)

Independent standards were used as QA/QC checks in all water chemistry and tissue analyses. Instrument calibration required an $r^2 > 0.99$ for the calibration curve. All samples were within our standard range (0.2 – 56.6 ng L^{-1}). Field replicates were randomly collected from three streams for each monthly water sampling, and two lab duplicates were analyzed for every set of samples. For tissue samples, duplicates were analyzed every tenth tissue digest. Replicates and duplicates were acceptable with < 5% variation. Due to the <1g of tissue available, replicates could not be taken from individual salamanders. We analyzed two replicates of DORM-3 fish protein (National Research Council of Canada) with each set of salamander tissue samples. The acceptable range of total mercury concentrations for DORM-3 was 322 ng g^{-1} to 442 ng g^{-1}. Our DORM-3 samples averaged 367.8 ± 5.4 ng g^{-1} within a range of 323.1 ng g^{-1} to 434.7 ng g^{-1}.

Statistical Analysis

Total mercury concentrations in northern two-lined salamanders were analyzed using a nested ANOVA to assess the differences in total mercury concentrations between the adult and larval life stages and among streams. Life stage of the salamanders, stream, and the interaction term between life stage and stream were fixed effects in the model. We included the seasonal samplings as a random effect in the model to account for pseudo-replication within the data set. Factors used in the ANOVA were tested for normality using the Lilliefors test of normality, prior to analysis. We transformed total mercury data using a ¼ root transformation to address non-normality of data. This transformation provides the best balance between normality of residuals in the model and homogeneity of variances. The transformed residuals passed Lilliefors test of normality ($P < 0.05$). Transformed total mercury concentrations were also tested for correlation with salamander weight, snout-vent length (SVL), and length using Pearson Product-Moment Correlation tests.

The mean values for the monthly stream water chemistry data were then used in a Pearson Product-Moment Correlation analysis with the mean total mercury concentrations for each life stage at each stream. All statistical analyses were performed using the Linear and Nonlinear Mixed Effects Models (nlme) package in the R-Project (version 2.12) (R Development Core Team 2009, Pinheiro et al. 2011).

RESULTS

Salamander Weight, Length, and SVL

The overall mean weight for adult northern two-lined salamanders was 0.79 ± 0.04 g within a range of 0.15 g to 1.91 g. Across individual streams, mean weight ranged from 0.52 ± 0.05 g at Bear Pen to 1.03 ± 0.10 g at Monroe Run. The overall mean weight for larvae was 0.19 ± 0.01 g within a range of 0.16 g to 0.69 g. Across individual streams, larvae ranged in mean weight from 0.10 ± 0.03 g at Little Savage River to 0.26 ± 0.03 g at Mill Run.

The overall mean SVL for adults was 35.05 ± 0.61 mm within a range of 21.0 mm to 48.2 mm. Across individual streams, mean SVL ranged from 30.78 ± 1.00 mm at Bear Pen to 38.48 ± 1.09 mm at Monroe Run. The overall mean SVL for larvae was 19.89 ± 0.34 mm within a range of 9.9 mm to 32.4 mm. Across individual streams, the mean SVL ranged from 16.98 ± 1.74 mm at Little Savage River to 21.21 ± 0.80 mm at Mill Run.

The overall mean total length was 75.65 ± 1.67 mm within a range of 40.5 mm to 117.8 mm (not including 6 individuals with missing tails). Across individual streams, mean total length ranged from 63.27 ± 2.62 mm at Bear Pen (not including 3 individuals missing tails) to 84.02 ± 3.33 mm at Monroe Run (not including 1 individual missing its tail). The overall mean total length for larvae was 35.28 ± 0.69 mm within a range of 17.5 mm to 61.5 mm. Across individual streams, mean total lengths ranged from 29.06 ± 3.42 mm at Little Savage River to 38.62 ± 1.77 mm at Mill Run.

Mercury Concentrations in Adult and Larval Northern Two-Lined Salamanders

Across all streams the mean total mercury concentration in adult northern two-lined salamanders was 29.54 ± 1.30 ng g^{-1} within a range of 10.92 ng g^{-1} to 73.78 ng g^{-1}. Across individual streams mean total mercury concentrations in adults ranged from 19.36 ± 1.76 ng g^{-1} at Mill Run to 40.97 ± 2.69 ng g^{-1} at Mud Lick

In larval northern two-lined salamanders the mean total mercury concentration was 22.08 ± 1.48 ng g^{-1} within a range of 0.64 ng g^{-1} to 92.56 ng g^{-1}. Across individual streams mean total mercury concentrations ranged from 6.73 ± 0.72 ng g^{-1} at Bear Creek to 42.20 ± 3.55 ng g^{-1} at Mud Lick.

The enhancement of total mercury concentrations from larval northern two-lined salamanders to adults varied across sites. The overall mean enhancement ratio (mean adult total mercury/mean larval total mercury) was 1.74. The ratio ranged from 0.97 at Mud Lick to 4.12 at Bear Creek (Table 5).

Mean total mercury concentrations in northern two-lined salamanders were significantly affected by life stage (F = 28.8466, df = 1,234, P <0.0001), stream (F = 7.2135, df = 6,11, P = .0026), and the life stage stream interaction term (F = 2.9908, df = 6,234, P = .0078). Mean total mercury concentrations in adult and larval northern two-lined salamanders were not statistically different at Mud Lick (adult: 40.97±2.69 ng g^{-1}, larvae: 42.20±3.55 ng g^{-1}) and Little Savage River (adult: 36.53±4.34 ng g^{-1}, larvae: 35.91±4.53 ng g^{-1}). For the other five study streams adult northern two-lined salamanders had significantly more total mercury than larval northern two-lined salamanders (Fig. 2, 3).

Mercury Concentrations in Egg Masses

Total mercury concentrations of 9.21 ng g^{-1} and 9.33 ng g^{-1} were in egg masses taken from one gravid female at both Mill Run and Monroe Run, respectively. These egg masses weighed 0.08 g and 0.34 g and contained 15 and 22 individual eggs resulting in 0.61 ng g^{-1} and 0.42 ng g^{-1} total mercury per egg, respectively.

Mercury and DOC in Streams

Total mercury, methyl mercury, and DOC varied across streams. Mean stream total mercury concentrations ranged from 0.52 ± 0.06 ng L^{-1} at Mill Run to 2.25 ± 0.26 ng L^{-1} at Little Savage River. Mean methyl mercury concentrations ranged from 0.06 ± 0.01 ng L^{-1} at Mill Run to 0.21 ± 0.05 ng L^{-1} at Little Savage River. Mean DOC concentrations ranged from 0.51 ± 0.04 mg L^{-1} at Bear Creek to 3.51 ± 0.24 mg L^{-1} at Little Savage (Table 6).

Correlation Analyses

In adults and larvae, body weight (A: r = 0.098, P = 0.3033, L: r = -0.071, P = 0.3951), length (A: r = -0.023, P = 0.8096, L: r = 0.020, P = 0.8150) and SVL (A: r = 0.143, P = 0.340, L: r = 0.073, P = 0.3818) were not significantly correlated with root transformed mercury concentrations (Table 7). Stream methyl mercury concentration was positively correlated with total mercury concentrations in adult (r = 0.756, P = 0.0491) and larval (r = 0.786, P = 0.0361) northern two-lined salamanders. DOC was positively correlated with total mercury concentrations in larval northern two-lined salamanders (r = 0.768, P = 0.439) (Table 8).

DISCUSSION

The weights and SVL of our adult and larval northern two-lined salamanders were similar to those reported in other studies (Bank et al. 2005; Lannoo 2005). Lannoo (2005) reported mean SVL of 29 to 41 mm for adult northern two-lined salamanders from populations throughout the range, from Nova Scotia to northern Virginia. In comparison, our adult salamanders ranged in mean SVL from 30.78 ± 1.00 mm to 38.48 ± 1.09 mm (Table 4). Bank et al. (2005) reported mean larval wet weights that ranged from 0.11 ± 0.02 g to 0.33 ± 0.02 g and mean larval SVL that ranged from 20 ± 0.07 mm to 27 ± 0.06 mm. Our mean larval weights ranged from 0.10 ± 0.03 g to 0.26 ± 0.03 g and our mean larval SVL ranged from 16.98 ± 1.74 mm to 21.21 ± 0.80 (Table 4). Our mean SVL measurements and those of Bank et al. (2005) were less than the mean SVL at time of metamorphosis of 30 mm (Lannoo 2005), illustrating that our sampled populations and those sampled by Bank et al. (2005) have a larval period of normal length and normal growth. Thus, our salamanders were typical of those from other stream ecosystems and are likely to be representative of populations in Garrett County, MD.

There was considerable variation in the total mercury concentrations in adult and larval northern two-lined salamanders. Total mercury concentrations in individual adult salamanders ranged from 10.92 ng g^{-1} at Mill Run to 73.78 ng g^{-1} at Poplar Lick. The median mercury concentration in adults was 26.07 ng g^{-1} and only 10 of 113 adult northern two-lined salamanders had total mercury concentrations exceeding 50 ng g^{-1}. Total mercury concentrations in larvae ranged from 0.64 ng g^{-1} at Bear Creek to 92.56 ng g^{-1} at Poplar Lick. The median total mercury concentration in larvae was 15.80 ng g^{-1} and only 10 of 146 had total mercury concentrations of greater than 50 ng g^{-1}.

Bank et al. (2005) reported mean total mercury concentrations in larval northern two-lined salamanders from Acadia National Park of 66.1±3.4 ng g^{-1} and from Shenandoah National Park of 26.8±1.8 ng g^{-1}. The mean concentration from Shenandoah is closer to our mean total mercury concentrations in larvae (22.08 ± 1.48 ng g^{-1}) than the mean larval concentration from Acadia. This may be explained by greater geographic and geologic similarities between our streams in western Maryland and those in Shenandoah National Park, Virginia.

The life cycle of northern two-lined salamanders contributes significantly to their mercury burden. During late spring, eggs are laid on the underside of rocks and other cover items in streams. Our limited data on the mercury concentrations in eggs suggests that maternal transfer of mercury is 0.42 ng g^{-1} to 0.61 ng g^{-1} per egg. These eggs hatch in 4-10 weeks into fully aquatic larvae. They can remain in the aquatic larval stage for up to 3 years (Lannoo 2005; Petranka 1998). The diet of larval northern two-lined salamanders includes many common aquatic macro-invertebrates and periphyton (Lannoo 2005; Petranka 1984). This diet has been shown to be a significant source of mercury for fish and is likely a significant source of mercury for larval salamanders (Cremona et al. 2008; Kelly et al. 2006; Prepas et al. 2005; Zizek et al. 2007). Our larval northern two-lined salamanders had mean total mercury concentrations around 40 to 50 times greater than the total mercury concentrations in eggs (0.42 to 0.61 ng g^{-1} vs. 22.08 ± 1.48 ng g^{-1}). The lack of significant maternal transfer of mercury suggests that the majority of mercury accumulated by larval northern two-lined salamanders is obtained from stream food sources.

As adults, northern two-lined salamanders consume a diet of mostly terrestrial macroinvertebrates and some aquatic benthic macroinvertebrates (Lannoo 2005; Petranka 1998). Assuming this terrestrial diet would contain lower mercury concentrations than the aquatic diet of the larvae, we hypothesized that adult northern two-lined salamanders would have lower total mercury concentrations than larval salamanders. The assumption that terrestrial macroinvertebrates would contain lower concentrations of mercury was based on a spatial separation from aquatic environments where mercury methylation occurs. However, Rimmer et al. (2010) reported high concentrations of total mercury in terrestrial arthropods in a montane forest in Vermont, with reported concentrations ranging from 8 ng g^{-1} (*Hemiptera sp.*) to 176 ng g^{-1} (*Araneae sp.*). Mercury deposition rates in Vermont are comparable to those in our study area (Miller et al. 2005). Therefore, it is reasonable to assume that the arthropods being consumed by adult northern two-lined salamanders in our study would exhibit similar concentrations of mercury to those reported in the Rimmer et al. study.

We found adult northern two-lined salamanders in five streams had significantly higher concentrations of total mercury than larval northern two-lined salamanders. At these five streams the enhancement ratio ranged from 1.15 at Poplar Lick to 4.12 at Bear Creek. This large range of enhancement ratios further emphasizes the large variation in mercury concentrations across the sites. The large enhancement ratio at Bear Creek may be influenced by the small sample size of adults at this stream, but it does indicate a potentially large difference in mercury uptake between the adults and larvae. The increase in total mercury concentration from larvae to adult and the data reported by Rimmer et al. suggests that the terrestrial diet of the adult northern two-lined salamanders may contain mercury comparable to the diets of the larvae, contrary to our original assumption. Therefore, the higher total mercury concentrations in the adult northern two-lined salamanders is likely related to their continued growth and age as a function of their continued consumption of high mercury prey.

At two streams, Mud Lick and Little Savage River, adults and larvae had statistically similar mean concentrations of total mercury. In addition, salamanders from these two streams had the highest concentrations of total mercury (Fig. 2). These two streams also had the highest concentrations of total and methyl mercury and DOC (Table 5). High concentrations of DOC have been shown to carry mercury in stream ecosystems (Balogh et al. 2003; Brigham et al. 2009). This is consistent with our hypothesis that streams with higher concentrations of mercury would have salamanders with higher concentrations of mercury. Methyl mercury and DOC concentrations in stream water were positively correlated with total mercury concentrations in adults and larvae, suggesting a strong link between salamander mercury accumulation and stream mercury cycling. This same relationship was also present in brook trout in western Maryland streams with higher methyl mercury concentrations (Castro et al. 2007).

At these higher mercury streams, we would expect to find higher concentrations of mercury in the prey of the adult and larval northern two-lined salamanders. This expectation is consistent with Castro et al. (2007) who reported that macroinvertebrates of *Heptagenidae sp.* and *Acroneuria sp.* in the Little Savage River have significantly higher mercury concentrations than those found in Monroe Run. Both of these macroinvertebrates are food sources for larval northern two-lined salamanders. This suggests that larval northern two-lined salamanders at Little Savage River may be consuming significantly more mercury than larvae at other streams. Therefore, the larvae in Little Savage River would have higher total mercury concentrations than larvae in other streams, which is consistent with our data (Table 5). Despite having no data for macroinvertebrates at Mud Lick, it is possible that the larvae in Mud Lick are also consuming significantly more mercury than at other sites.

We were surprised to find no statistical difference between adult and larval total mercury concentrations at Mud Lick and Little Savage River. This may indicate that the mercury content in the aquatic diet available to the larvae is similar in magnitude to the mercury content of the terrestrial diet available to adults. This difference in food source mercury content may be due to the higher mercury concentrations in the aquatic macroinvertebrates of these streams.

CONCLUSION

The unique physiologies of stream salamanders, their complex life histories, and their presence in streams too shallow or intermittent to support fish may make them effective bioindicator species of mercury contamination in small stream reaches (Bank et al. 2005; Southerland 2004). Improving our knowledge of mercury dynamics in these salamanders will increase our understanding of the movement of mercury through the environment.

Our findings that, contrary to our original hypothesis, mercury concentrations in adult salamanders were higher than mercury concentrations in larval salamanders at five of seven streams may indicate that some of our original assumptions may be incorrect. The terrestrial diets of these adult salamanders may not be lower in mercury content than the aquatic diets of the larval salamanders as we assumed.

Our data supports our hypothesis that streams with higher total and methyl mercury concentrations would also have salamanders with higher total mercury concentrations. Higher stream mercury concentrations may indicate higher mercury concentrations in aquatic macroinvertebrates, which may result in higher mercury concentrations in northern two-lined salamanders as seen in our Mud Lick and Little Savage River streams.

Diet may play a large role in the variations in mercury concentration across populations of northern two-lined salamanders. Differences in the mercury pool in the food sources for adult and larval northern two-lined salamanders may explain a significant portion of the variation seen in the mercury concentrations between the two life stages. Further study is needed to identify the sources of mercury to northern two-lined salamanders. More information is also needed about adult and larval diets at specific locations, across stream differences in mercury in the aquatic and terrestrial food sources, and within stream differences between aquatic and terrestrial food sources. The results of this study indicate that terrestrial food webs may be higher in mercury than was previously assumed. Further study of mercury concentrations in stream salamanders will help to better understand the dynamics of mercury movement between stream and riparian ecosystems.

TABLES AND FIGURES

Table 1 Streams sampled in Garrett County, MD with number of salamanders collected.

Stream Name	Latitude	Longitude	Number of Salamanders	
			Adults	Larvae
Bear Creek	39.6503	-79.2903	2	15
Bear Pen Run	39.5626	-79.1117	24	18
Little Savage River	39.6169	-79.0249	10	5
Mud LicK	39.6461	-79.0257	30	29
Monroe Run	39.5553	-79.2166	16	19
Mill Run	39.7135	-79.3781	20	30
Poplar Lick	39.6385	-79.1175	11	30
Total			113	146

Table 2 Watershed composition and land use of streams sampled in Garrett County, MD. Land use data is calculated from the National Land Cover Database (NLCD) 2006 data set.

	Land Use (%)					Forest type (%)			Total Area
Stream	**Wet.**	**Ag.**	**Dev.**	**For.**	**Bare**	**Decid.**	**Ever.**	**Mixed**	**(Ha)**
Bear Creek	0	0.82	2.43	96.59	0.17	80.13	16.46	0	95
Bear Pen	0	9.88	1.57	88.07	0.48	84.34	2.44	1.29	867
L. Sav. River	0.14	0.3	1.95	95.21	0.21	70.96	22.47	1.77	555
Mud Lick	0	29.1	7.73	61.92	1.2	42.92	18.07	0.93	1432
Monroe Run	0	2.68	4.8	92.46	0.05	87.69	3.97	0.8	1201
Mill Run	0	15.61	9.42	74.28	0.59	69.72	3.86	0.7	4365
Poplar Lick	0.18	5.46	3.36	89.74	0.9	67.68	19.81	2.26	2139

Table 3 List of methods used in analysis of water chemistry.

	Procedure for Sample Preparation	**Method of Analysis**
Dissolved Organic Carbon	EPA Method 415.3	Ultraviolet Absorbance at 254 nm
Total Suspended Solids	ESS Method 340.3	Filtration and Drying to 103-105 C
Major Anions (Cl^-, NO_3^-, SO_4^{2-})	EPA Method 300 Rev 2.1	Ion Chromatography
Acid Neutralizing Capacity		Gran Analysis Technique
pH	EPA Method 150.1	Electrometric Determination
Total Mercury	EPA Method 1631	Cold Vapor Atomic Fluorescence Spectrophotometry (CVAFS)
Methyl Mercury	EPA Method 1630	Ethylation and Preconcentration Purge and Trap Techniques

Table 4 Mean weight (±SE), length (± SE), and SVL (±SE) of adult and larval northern two-lined salamanders sampled from streams in Garrett County, MD.

	Mean Weight (g)		Mean SVL (mm)		Mean Total Length (mm)		
Site	**Adult**	**Larvae**	**Adult**	**Larvae**	**Adult**	**Larvae**	**A. Missing Tails**
Bear Creek	0.99 ± 0.02	0.14 ± 0.02	39.8 ± 1.3	18.89 ± 0.90	81.55 ± 0.55	33.67 ± 1.64	0
Bear Pen	0.52 ± 0.05	0.18 ± 0.02	30.78 ± 1.00	19.65 ± 0.92	63.27 ± 2.62	35.03 ± 1.74	3
Little Savage River	0.80 ± 0.12	0.10 ± 0.03	35.57 ± 1.94	16.98 ± 1.74	75.27 ± 4.89	29.06 ± 3.42	0
Mud Lick	0.78 ± 0.06	0.17 ± 0.02	35.55 ± 1.26	19.04 ± 0.83	75.45 ± 3.28	33.76 ± 1.62	1
Monroe Run	1.03 ± 0.10	0.20 ± 0.02	38.48 ± 1.09	20.77 ± 0.93	84.02 ± 3.33	36.75 ± 1.91	1
Mill Run	0.84 ± 0.10	0.26 ± 0.03	35.53 ± 1.69	21.21 ± 0.80	80.71 ± 4.68	38.62 ± 1.77	1
Poplar Lick	0.93 ± 0.14	0.16 ± 0.02	35.74 ± 1.97	19.96 ± 0.73	78.85 ± 5.29	34.47 ± 1.31	0
Overall Mean	0.79 ± 0.04	0.19 ± 0.01	35.05 ± 0.61	19.89 ± 0.34	75.65 ± 1.67	35.28 ± 0.69	6

Table 5 Table of mean total mercury concentrations in adults and larval northern two-lined salamanders (±SE) collected from streams in Garrett County, MD and the ratio of adult to larvae total mercury.

	Mean Total Mercury (ng g^{-1})		Adult/Larvae
Stream	**Adult**	**Larvae**	**THg**
Bear Creek	27.70 ± 4.69	6.73 ± 0.72	4.12
Bear Pen	24.54 ± 1.65	13.37 ± 2.65	1.84
Little Savage River	36.53 ± 4.34	35.90 ± 4.53	1.02
Mud Lick	40.97 ± 2.69	42.20 ± 3.55	0.97
Monroe Run	22.87 ± 1.56	18.30 ± 1.64	1.25
Mill Run	19.36 ± 1.76	10.41 ± 0.82	1.86
Poplar Lick	31.45 ± 4.64	27.30 ± 3.52	1.15
Overall Mean	29.54 ± 1.30	22.08 ± 1.48	1.74

Table 6 Mean dissolved organic carbon (DOC) (±SE), total mercury (THg) (±SE), methyl mercury (MeHg) (±SE), and methylation efficiency (MeHgeff) in streams in Garrett County, MD.

Stream	Mean DOC mg L^{-1}	Mean THg ng L^{-1}	Mean MeHg ng L^{-1}	Mean MeHgeff Percent
Bear Creek	0.51 ± 0.04	0.78 ± 0.12	0.07 ± 0.01	10.1
Bear Pen	0.84 ± 0.06	0.66 ± 0.05	0.09 ± 0.02	12.59
Little Savage River	3.51 ± 0.24	2.25 ± 0.21	0.21 ± 0.05	9.43
Mud Lick	2.06 ± 0.25	1.00 ± 0.10	0.15 ± 0.03	14.56
Monroe Run	0.81 ± 0.06	0.60 ± 0.06	0.09 ± 0.02	14.71
Mill Run	1.02 ± 0.07	0.52 ± 0.06	0.06 ± 0.01	12.26
Poplar Lick	0.95 ± 0.06	0.76 ± 0.09	0.08 ± 0.02	10.74
Overall Mean	1.39 ± 0.14	0.94 ± 0.08	0.11 ± 0.01	12.06 ± 0.88

Table 7 Pearson product-moment Correlation table of ¼ root transformed total Hg data, weight, SVL, length. Significant correlations ($P < 0.05$) are marked with an asterisk.

	¼ Root Total Hg	**Weight**	**SVL**
Adults			
Weight	0.098		
SVL	0.143	0.947*	
Length	-0.023	0.232*	0.295*
Larvae			
Weight	-0.071		
SVL	0.073	0.884*	
Length	0.02	0.913*	0.952*

Table 8 Pearson product-moment correlation table of ¼ root transformed mean total mercury concentrations in adult and larval northern two-lined salamanders and mean water chemistry factors. Significant correlations ($P < 0.05$) are marked with an asterisk.

	¼ Root Total Hg	**pH**	**ANC**	**THg**	**MeHg**	**DOC**	**TSS**	**Cl^{-1}**	**NO_3^{-1}**
Adult									
pH	-0.537								
ANC	-0.211	0.843*							
THg	0.639	-0.948*	-0.71						
MeHg	0.756*	-0.898*	-0.567	0.931*					
DOC	0.688	-0.888*	-0.517	0.935*	0.963*				
TSS	0.333	-0.437	-0.695	0.354	0.24	0.187			
Cl^{-1}	-0.042	0.394	0.748	-0.265	-0.107	0.036	-0.611		
NO_3^{-1}	-0.625	0.437	0.407	-0.411	-0.462	-0.275	-0.278	0.652	
SO_4^{-2}	-0.625	0.437	0.407	-0.411	-0.462	-0.275	-0.278	0.652	1.000*
Larvae									
pH	-0.592								
ANC	-0.173	0.843*							
THg	0.611	-0.948*	-0.71						
MeHg	0.786*	-0.898*	-0.567	0.931*					
DOC	0.768*	-0.888*	-0.517	0.935*	0.963*				
TSS	0.105	-0.437	-0.695	0.354	0.24	0.187			
Cl^{-1}	0.121	0.394	0.748	-0.265	-0.107	0.036	-0.611		
NO_3^{-1}	-0.519	0.437	0.407	-0.411	-0.462	-0.275	-0.278	0.652	
SO_4^{-2}	-0.519	0.437	0.407	-0.411	-0.462	-0.275	-0.278	0.652	1.000*

Figure 1 Map of streams sampled in Garrett County, MD within the Youghiogheny and Savage River Watersheds.

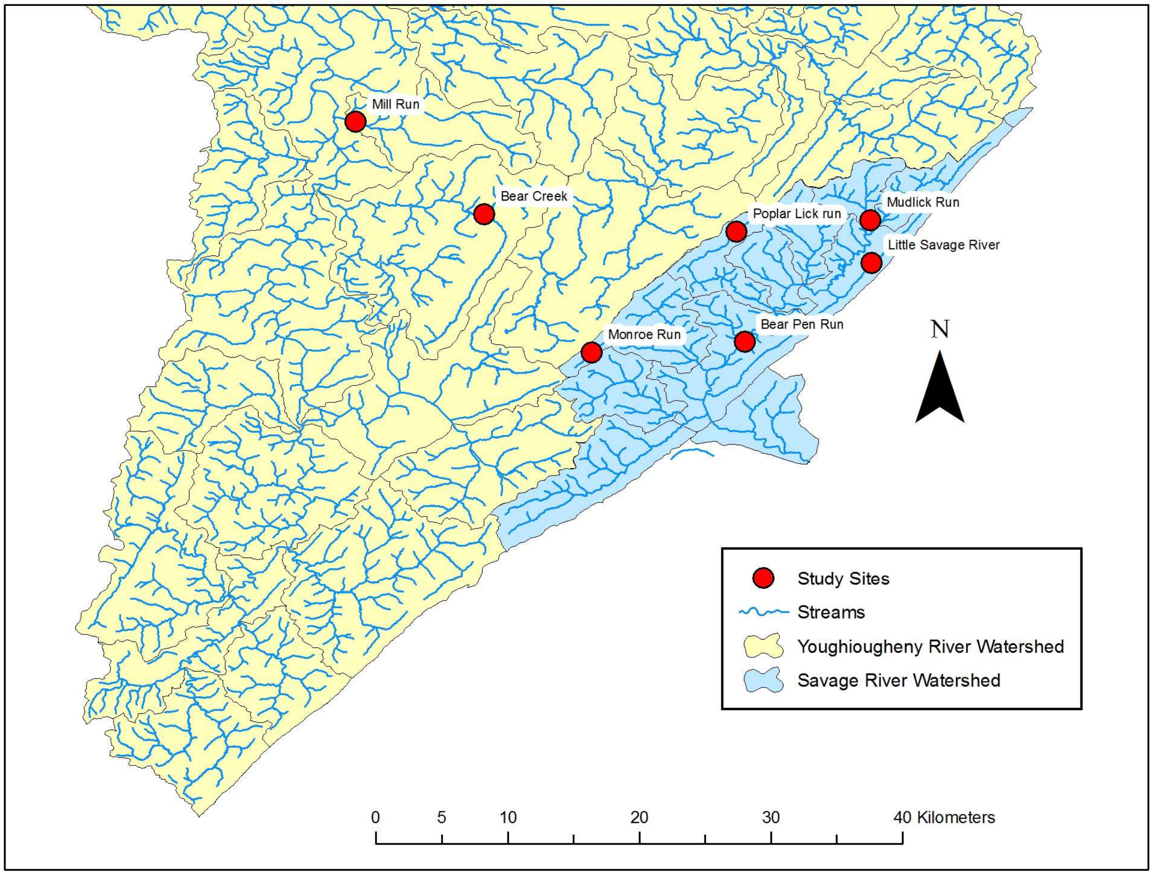


Figure 2 Mean Total Mercury Concentrations between adult and larval northern two-lined salamanders from Bear Creek (BC), Bear Pen (BP), Little Savage River (LSR), Mud Lick (ML), Monroe Run (MON), Mill Run (MR), and Poplar Lick (PL) in Garrett County, MD (±SE).

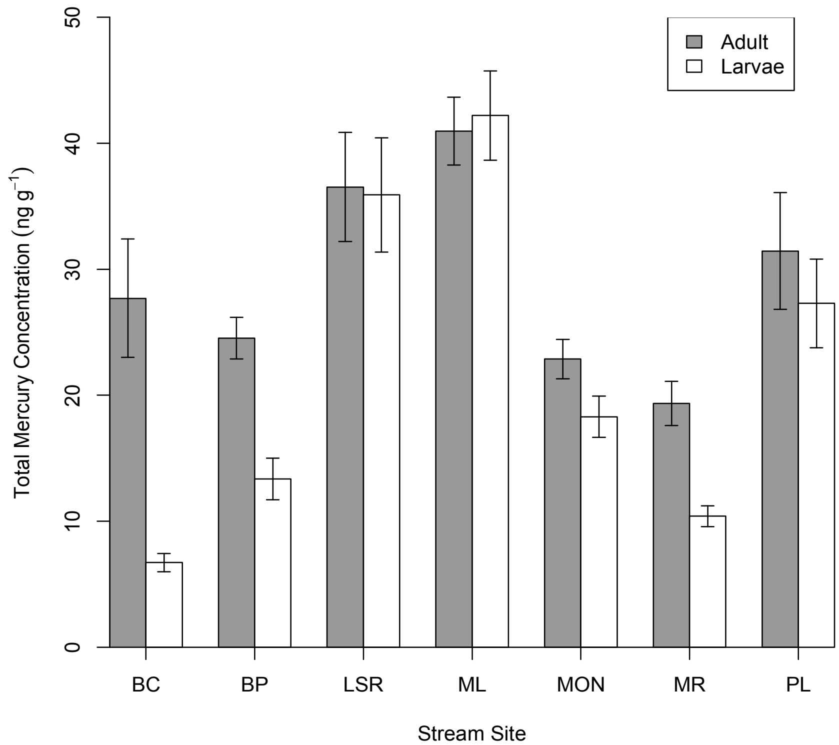
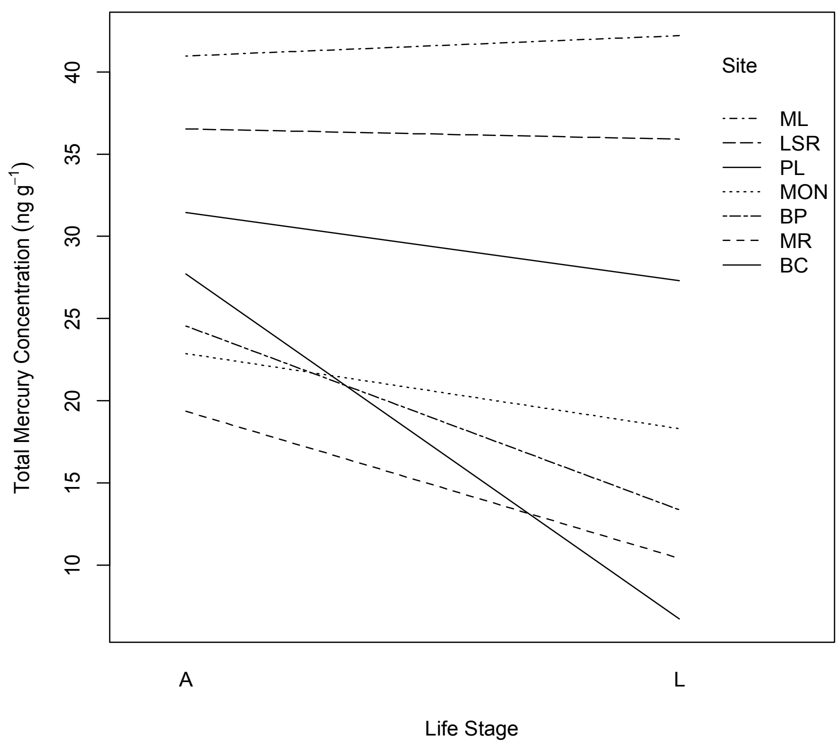


Figure 3 Interaction plot of life stage and stream site for mean total mercury concentrations in northern two-lined salamanders from Bear Creek (BC), Bear Pen (BP), Little Savage River (LSR), Mud Lick (ML), Monroe Run (MON), Mill Run (MR), and Poplar Lick (PL) in Garrett County, MD.



LITERATURE CITED

Bank MS, Loftin C, Jung R (2005) Mercury Bioaccumulation in Northern Two-Lined Salamanders from Streams in the Northeastern United States. Ecotoxicol 14: 181-191.

Ben-Ari E (2005) A New Piece in the Puzzle of Global Amphibian Declines. Biosci 55, 1: 96.

Bergeron CM, Bodinof C, Unrine J, Hopkins W (2010) Mercury Accumulation Along a Contamination Gradient and Nondestructive Indices of Bioaccumulation in Amphibians. Environ Toxicol Chem 29, 4: 980-988.

Balogh SJ, Yabing H, Offerman H, Meyer M, Johnson D (2003) Methylmercury in rivers draining cultivated watersheds. Sci Total Environ 304: 305-313.

Boone MD, James S (2003) Interactions of an Insecticide, Herbicide, and Natural Stressors in Amphibian Community Mesocosms. Ecol Appl 13, 3: 829-841.

Brigham ME, Wentz D, Aiken G, Krabbenhoft D (2009) Mercury Cycling in Stream Ecosystems. 1. Water Column Chemistry and Transport. Environ Sci Technol 43, 8: 2720-2725.

Burke JN, Bergeron C, Todd B, Hopkins W (2010) Effects of mercury on behavior and performance of northern two-lined salamanders (Eurycea bislineata). Environ Pollut 158: 3546-3551.

Castro MS, Hilderbrand R, Thompson J, Heft A, Rivers S (2007) Relationships Between Wetlands and Mercury in Brook Trout. Arch Environ Con Tox 52: 97-103.

Cremona F, Planas D, Lucotte M (2008) Assessing the importance of macroinvertebrate trophic dead ends in the lower transfer of methylmercury in littoral food webs. Can J Fish Aquat Sci 65, 9: 2043-2052.

Gilmour CG and Henry E (1991) Mercury Methylation in Aquatic Systems Affected by Acid Deposition. Environ Pollut 71: 131–69.

Hatch AC and Blaustein A (2003) Combined Effects of UV-B Radiation and Nitrate Fertilizer on Larval Amphibians. Ecol Appl 13, 4: 1083-1093.

Kelly EN, Schindler D, St. Louis V, Donald D, Vladicka K (2006) Forest Fire Increases Mercury Accumulation by Fishes via Food Web Restructuring and Increased Mercury Inputs. P Natl Acad Sci USA 103, 51: 19380-19385.

Lannoo M (2005) Amphibian Declines: The Conservation Status of United States Species. University of California Press, Berkeley and Los Angeles

Lenkowski JR, Reed J, Deininger L, McLaughlin K (2008) Perturbation of Organogenesis by the Herbicide Atrazine in the Amphibian Xenopus laevis. Environ Health Persp 116, 2: 223-230.

Mason RP, Laporte JM, Andres S (2000) Factors Controlling the Bioaccumulation of Mercury, Methylmercury, Arsenic, Selenium, and cadmium by Freshwater Invertebrates and Fish. Arch Environ Con Tox 38: 283-297.

Mendelson III JR (2006) Confronting Amphibian Declines and Extinctions. Science 313, 5783: 48.

Miller EK, VanArsdale A, Keeler JG, Chalmers A, Poissant L, Kamman NC, Brulotte R (2005) Estimation and mapping of wet and dry mercury deposition across northeastern North America. Ecotoxicology 14:53–70.

Moore CW, Cosby B, Galloway J, Castro M (2009) Relationship Between pH and Stream Water Total Mercury Concentrations in Shenandoah National Park. Water Air Soil Poll 201: 233-238.

Moore CW, Castro M, Brooks S (2011) Modified Bowen Ratio Fluxes of Gaseous Elemental Mercury in Western Maryland. Atmos Environ, In Revision.

Morel FM, Kraepiel A, Amyot M (1998) The Chemical Cycle and Bioaccumulation of Mercury. Annu Rev Ecol Syst 29: 543-566.

National Atmospheric Deposition Program / Mercury Deposition Network (NADP/MDN) (2004) NADP Program Office, Illinois State Water Survey, 2204 Griffith Drive, Champaign, IL 61820, http://nadp.sws.uiuc.edu/.

Peckenham JM, Kahl J, Nelson S, Johnson K, Haines T (2007) Landscape Controls on Mercury in Streamwater at Acadia National Park, USA. Environ Monit Assess 126: 97-104.

Pinheiro J, Bates D, DebRoy S, Sarkar D, and the R Development Core Team (2011) nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-98.

Petranka JW (1984) Ontogeny of the Diet and Feeding Behavior of *Eurycea bislineata* Larvae. J Herpetol 18, 1: 48-55.

Petranka JW (1998) Salamanders of the United States and Canada. Smithsonian Institution Press, Washington, DC

Prepas EE, Gabos S, Strachan W, Weiping Z (2005) Methyl mercury concentrations in macroinvertebrates and fish from burned and undisturbed lakes on the Boreal Plain. Can J Fish Aquat Sci 62, 9: 1963-1977.

R Development Core Team (2009) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Rimmer CC, Miller EK, McFarland KP, Taylor RJ, Faccio FD, (2010) Mercury bioaccumulation and trophic transfer in the terrestrial food web of a montane forest. Ecotoxicology 19: 697-709.

Rouse JD, Bishop C, Struger J, (1999) Nitrogen Pollution: An Assessment of Its Threat to Amphibian Survival. Environ Health Persp 107, 10: 799-803.

Rovito SM, Parra-Olea G, Vasquez-Almazan C, Papenfuss T, Wake D (2009) Dramatic declines in neotropical salamander populations are an important part of the global amphibian crisis. P Natl Acad Sci USA 106: 3231-3236.

Schiesari L, Grillitsch B, Grillitsch H (2007) Biogeographic Biases in Research and Their Consequences for Linking Amphibian Declines to Pollution. Conserv Biol 21, 2:465-471.

Shanley JB, Kamman N, Clair T, Chalmers A (2005) Physical Controls on Total and Methylmercury Concentrations in Streams and Lakes of the Northeastern USA. Ecotoxicol 14: 125-134.

Southerland MT, Jung R, Baxter D, Chellman I, Mercurio G, Volstad J (2004) Stream Salamanders as Indicators of Stream Quality in Maryland, USA. Appl Herpetol 2: 23-46.

Stokstad E (2004) Global Survey Documents Puzzling Decline of Amphibians. Science 306, 5695: 391.

Storrs SI and Kiesecker J (2004) Survivorship Patterns of Larval Amphibians Exposed to Low Concentrations of Atrazine. Environ Health Persp 112, 10:1054-1057.

Taylor B, Skelly D, Demarchis L, Slade M, Galusha D, Rabinowitz P (2005) Proximity to Pollution Sources and Risk of Amphibian Limb Malformation. Environ Health Persp 113, 11: 1497-1501.

Williams RN, Bos D, Gopurenko D, DeWoody J (2008) Amphibian Malformations and Inbreeding. Biol Letters 4: 549-552.

Zizek S, Horvat M, Gibicar D, Fajon V, Toman M (2007) Bioaccumulation of mercury in benthic communities of a river ecosystem affected by mercury mining. Sci Total Environ 377, 2: 407-415.

CHAPTER 2: MERCURY CONCENTRATIONS IN THREE SPECIES OF SALAMANDERS IN STREAMS OF GARRETT COUNTY, MARYLAND

ABSTRACT

The purpose of this study was to examine differences in total mercury concentrations among three species of stream salamanders across six streams in Garrett County, MD. We measured total mercury concentrations in adults of two species, the northern two-lined salamander (*Eurycea bislineata bislineata*) and the northern dusky salamander (*Desmognathus fuscus*) collected in April, July, and September 2010. We also measured total mercury concentrations in adults of the Allegheny Mountain dusky salamander (*Desmognathus ocrophaeus*) collected in July and September 2010. Averaged over all streams, adult northern two-lined salamanders had significantly higher mean total mercury concentrations (29.57 ± 1.32 ng g^{-1}) than adult northern dusky (20.95 ± 0.78 ng g^{-1}) and mountain dusky salamanders (22.84 ± 1.23 ng g^{-1}). Adult northern dusky and mountain dusky salamanders were not significantly different. This may be due to the longer larval period of the northern two-lined salamanders (24-36 months) compared to northern dusky (9-12 months) and Allegheny Mountain dusky salamanders (0-3 months). A longer larval period suggests that the northern two-lined salamanders were consuming a fully aquatic diet, which may be higher in mercury, for a longer time period. Mean tissue total mercury concentrations in northern dusky salamanders were more highly correlated with stream water methyl mercury concentrations ($r = 0.898$) than northern two-lined salamanders ($r = 0.776$) and Allegheny Mountain dusky salamanders

(r = 0.748). Mercury concentrations in the tissues the three species were highest at two study streams. These two streams also had the highest concentrations of total and methyl mercury as well as DOC, which may indicate higher concentrations of mercury in the prey species of the salamanders. These variations indicate the importance further study into mercury concentrations in stream salamanders.

INTRODUCTION

Fish that are consumed by humans have long been a focus of mercury research, but little is known about the concentrations of mercury that can be measured in smaller animals such as salamanders that occupy lower trophic levels. Salamanders and other amphibians are unique, both in their physiology and in their life cycles. Aquatic egg and larval stages metamorphose into terrestrial adults in many species. Other species spend their entire lives in the water, some entirely on land. There has only been one study measuring mercury concentrations in streams in western Maryland, which focused on adult and larval northern two-lined salamanders (Castro et al. 2018). This study measured higher concentrations in adult salamanders than the larvae at most streams, with two streams having statistically similar concentrations between the adults and larvae. Several other recent studies have identified significant mercury concentrations in salamanders and other amphibians in streams with similar mercury concentrations to those in Western Maryland (Bank et al. 2005) and in contaminated streams with significantly higher mercury concentrations (Bergeron et al. 2010; Burke et al. 2010). With the exception of Bergeron et al. (2010), these studies have all focused on one species, the northern two-lined salamander. The unique physiology and life history of salamanders make them effective indices of pollution, including mercury contamination, in stream ecosystems (Bank et al. 2005, Southerland 2004). In order to better understand the dynamics of mercury movement throughout stream habitats it is important to gain a greater knowledge of the differences in mercury concentrations among different species of stream salamander.

This study compared mercury concentrations in adults of three species of stream salamander collected from six streams in Garrett County Maryland. The species examined here include northern dusky (*Desmognathus fuscus*), northern two-lined (*Eurycea bislineata bislineata*), and Allegheny Mountain dusky salamanders (*D. ocrophaeus*). These three species are common streamside inhabitants found along rocky streams. Northern dusky salamanders are found under cover items rarely more than a meter from the stream edge. Northern two-lined salamanders are also often found under rocks and other cover items at the stream edge, though they do disperse into the surrounding uplands and can be found several meters from the stream edge. Allegheny Mountain dusky salamanders share habitat with the other two species but can be found farther from streams and are often encountered in the upland areas surrounding streams. The extensive, overlapping ranges of these three species and their shared habitat preferences make them good candidates to be used to compare mercury contamination and mercury bioaccumulation across wide regions (Lannoo 2005, Petranka 1998).

Northern dusky salamanders and northern two-lined salamanders have similar life histories. Both species have fully aquatic larvae, but the time spent in the larval stage differs between the two species. Northern two-lined salamanders can remain as larvae for up to three years, while the larval period of the northern dusky salamanders last for nine to twelve months. Both northern dusky and northern two-lined larvae feed on a wide variety of small aquatic macroinvertebrates. The aquatic diets of the larval stage of these two species expose them to potentially high concentrations of mercury. Adults of these species have differing diets, however, which potentially expose them to different amounts of mercury throughout their lives. Northern dusky adults consume a mixture of aquatic and terrestrial macroinvertebrates as well as the molted skins and larvae of other northern dusky salamanders. Adult northern two-lined salamanders also consume a mixture of aquatic and terrestrial macroinvertebrates, with a bias more towards terrestrial prey (Petranka 1998, Lannoo 2005). Terrestrial macroinvertebrates can range significantly in tissue total mercury concentrations (Rimmer et al. 2010). It was assumed that the more terrestrial diet of the northern two-lined salamanders would expose them to lower concentrations of mercury than the northern dusky salamanders. Though the previous research by Castro et al. (2018) and the work by Rimmer et al. indicate that this assumption may not be correct.

Allegheny Mountain dusky salamanders are distinctly more terrestrial than the other species in this study. Their eggs are laid under moist logs, and these salamanders have a short-lived larval stage of 0-3 months. Larval Allegheny Mountain dusky salamanders can remain on land if conditions are moist enough. Both adults and larvae eat mostly terrestrial macroinvertebrates (Lannoo 2005, Petranka 1998). The lack of a long aquatic larval period potentially exposes the Allegheny Mountain dusky salamanders to lower concentrations of mercury.

Aquatic macroinvertebrates are likely an important source of mercury for all three species of salamander (Prepas et al. 2005, Castro et al. 2006, Kelly et al. 2006, Zizek et al. 2007, Cremona et al. 2008). However, the impact of terrestrial diets on mercury accumulation is not well understood.

The objective of the study was to identify differences in mercury concentrations between species of stream salamander. From the data of Bank et al. (2005) and Bergeron et al. (2010), as well as lifecycle similarities and differences between these three species, we hypothesized that because of their longer larval period northern two-lined salamanders would accumulate significantly more mercury than the other two species. We also hypothesized that because of their terrestrial lifestyles and diets, the Allegheny Mountain dusky salamanders would accumulate significantly less mercury than the other two species.

METHODS

Study Streams

We studied salamanders in six first-order streams in western Maryland. Five streams were located in the Savage River watershed and one stream was located in the Youghiogheny River watershed (Fig. 4). Mean pH and ANC in these streams ranged from 5.98 to 6.70 and from 43.9 $\mu eq\ L^{-1}$ to 467.8 $\mu eq\ L^{-1}$ respectively. These small streams drained watersheds dominated by forest (62% to 97%) and agriculture (<1% to 29%). Watershed area ranged from 555 ha at Little Savage River to 4,365 ha at Mill Run. However, most watersheds were less than 2,200 ha (Table 10). Our study streams and watersheds are similar to those found throughout the Appalachian Plateau region of western Maryland.

Field Sampling

We collected salamanders in April, July, and September of 2010 using visual encounter surveys. To collect adult salamanders, cover objects were overturned within 2 m of the stream edge. Survey sampling for salamanders lasted for 2 to 6 hours at each stream. Salamanders were collected using a small aquarium dip net. After capture, they were transported in glass jars to the Appalachian Laboratory (AL) in Frostburg, MD. We attempted to collect 10 adult salamanders per species per stream for each sampling. The total number of samples at individual streams ranged from 6 to 20 for Allegheny Mountain dusky salamanders, 11 to 30 for northern two-lined salamanders, and 25 to 30 for northern dusky salamanders due to time, weather, and other sampling constraints (Table 9). Dry conditions during summer likely reduced the number of individuals collected.

Water Chemistry

Stream water was sampled monthly from April through December 2010 with the exception September. Measurements of water temperature, specific conductivity, and pH were made *in situ* using a Hydrolab Quanta model sonde, which was calibrated prior to each sampling. Grab samples of stream water were collected in acid cleaned high density polyethylene (HDPE) bottles for lab analysis of closed pH, ANC, total and methyl mercury, dissolved organic carbon (DOC), total suspended solids (TSS), chloride (Cl^{-1}), nitrate (NO_3^{-1}), and sulfate (SO_4^{-2}). Water samples were filtered through 0.45 µm Whatman glass fiber filters for TSS and DOC. Mercury samples were collected in ultra clean 1 L Teflon bottles and double Ziploc bagged using the “clean hand/dirty hand” technique (EPA method 1639). All samples were placed on ice and transported in coolers to the AL for analysis. Samples were stored at -4ºC and were analyzed within 1 month of collection using EPA protocols (Table 11).

Total Mercury in Salamanders

At AL, the live salamanders were euthanized in a buffered 10 g L^{-1} solution of methane tricainesulfonate (MS-222). Once euthanized, salamanders were measured for weight, total length, and snout-vent length (SVL). Salamanders were then double bagged and frozen at -22ºC until analyzed. All analyses were completed within 3 months of sample collection. Prior to analysis, salamanders were thawed and allowed to reach room temperature. Afterwards, whole salamanders were digested overnight in an acid solution of 70% concentrated sulfuric acid and 30% concentrated nitric acid. The following day, 100-150 µL of digestion solution and 250 µL of bromine mono-chloride (BrCl) were added to 50 mL of distilled de-ionized water in separate 65 mL ultra-clean FEP Teflon auto-analyzer vials. The total mercury concentration in this solution was measured using cold vapor atomic fluorescence spectroscopy (CVAFS) in a Tekran 2600 CVAFS mercury analysis system in the class 100 clean room at AL (EPA Method 1631).

Quality Assurance/Quality Control (QA/QC)

Independent standards were used as QA/QC checks in all water chemistry and tissue analyses. Instrument calibration required an $r^2 > 0.99$ for the calibration curve. All samples were within our standard range (0.2 – 56.6 ng L^{-1}). Field replicates were randomly collected from three streams for each monthly water sampling, and two lab duplicates were analyzed for every set of samples. For tissue samples, duplicates were analyzed every tenth tissue digest. Replicates and duplicates were acceptable with < 5% variation. Due to the <1g of tissue available, replicates could not be taken from individual salamanders. We analyzed two replicates of DORM-3 fish protein (National

Research Council of Canada) with each set of salamander tissue samples. The acceptable range of total mercury concentrations for DORM-3 was 322 ng g^{-1} to 442 ng g^{-1}. Our DORM-3 samples averaged 367.8 ± 5.4 ng g^{-1} within a range of 323.1 ng g^{-1} to 434.7 ng g^{-1}.

Statistical Analysis

Total mercury concentrations in salamanders were analyzed using a nested ANOVA to assess the differences in total mercury concentrations among species and among streams. Salamander species, stream, and the interaction term between species and stream were fixed effects in the model. We included the seasonal samplings as a random effect in the model to account for pseudo-replication within the data set. Factors used in the ANOVA were tested for normality using the Lilliefors test of normality, prior to analysis. We transformed total mercury data using a ¼ root transformation to address non-normality of data. This transformation provided the best balance between normality of residuals in the model and homogeneity of variances. The transformed residuals passed Lilliefors test of normality ($P < 0.05$). Transformed total mercury concentrations were also tested for correlation with salamander weight, snout-vent length (SVL), and length using Pearson Product-Moment Correlation tests.

Salamander weight, length and SVL were analyzed in separate 2-way ANOVA's. The terms in the model were salamander species, stream site, and the interaction between species and site. The interaction terms were non-significant and were subsequently removed from the models.

The mean values for the monthly stream water chemistry data were then used in a Pearson Product-Moment Correlation analysis with the mean total mercury concentrations for each species at each stream. All statistical analyses were performed using the Linear and Nonlinear Mixed Effects Models (nlme) package in the R-Project (version 2.12) (R Development Core Team 2009, Pinheiro et al. 2011).

RESULTS

Salamander Weight, SVL, and Length

The overall mean weight for northern dusky salamander was 2.31 ± 0.09 g within a range of 0.28 g and 5.69 g, which is significantly greater than the mean weights for the other two species ($P < 0.05$). The overall mean weight for northern two-lined salamanders was 0.79 ± 0.04 g within a range of 0.15 g to 1.91 g, and the overall mean weight for Allegheny Mountain dusky salamanders was 0.99 ± 0.04 g within a range of 0.12 g to 2.01 g. Northern two-lined salamanders and Allegheny Mountain dusky salamanders had statistically similar weights.

Northern dusky salamanders also significantly longer mean SVL than the other two species ($P < 0.05$). The overall mean SVL for northern dusky salamanders was 45.58 ± 0.61 mm within a range of 23.6 mm to 62.6 mm. The overall mean SVL for northern two-lined salamanders was 34.96 ± 0.62 mm within a range of 21.0 mm to 48.2 mm, and the overall mean SVL for Allegheny Mountain dusky salamanders was 36.01 ± 0.63 mm within a range of 17.8 mm to 47.2 mm. Northern two-lined salamanders and Allegheny Mountain dusky salamanders had statistically similar SVL.

Northern Dusky salamanders had significantly longer total lengths than the other two species at 89.06 ± 1.22 mm within a range of 46.5 mm to 124.2 mm (not including 15 individuals with missing tails) ($P < 0.05$). The overall mean total length for northern two-lined salamanders was 75.53 ± 1.70 mm within a range of 40.5 mm to 117.8 mm (not including 6 individuals with missing tails). The overall mean total length for Allegheny Mountain dusky salamanders was 76.03 ± 1.51 mm within a range of 34.3 mm to 102.3 mm (not including 5 individuals missing tails). Northern two-lined salamanders and Allegheny Mountain dusky salamanders were statistically similar in total length.

Total Mercury Concentrations in Adult Salamanders

Total mercury concentrations in adult salamanders were significantly different among species ($F = 30.905$, $P < 0.001$) and streams ($F = 8.740$, $P = 0.0011$). Mean total mercury concentrations in northern dusky salamanders (20.95 ± 0.78 ng g^{-1}) and Allegheny Mountain dusky (22.84 ± 1.23 ng g^{-1}) were significantly lower than northern two-lined salamanders (29.57 ± 1.32 ng g^{-1}) ($P < 0.0001$), but not different from each other across all streams ($P = 0.145$). There was no significant interaction between species and stream, so the interaction term was removed from the model.

Mercury and DOC in Streams

Total mercury, methyl mercury, and DOC concentrations varied across streams. Mean stream total mercury ranged from 0.52 ± 0.06 ng L^{-1} at Mill Run to 2.25 ± 0.26 ng L^{-1} at Little Savage River. Mean methyl mercury ranged from 0.06 ± 0.01 ng L^{-1} at Mill Run to 0.21 ± 0.05 ng L^{-1} at Little Savage River. Mean DOC ranged from 0.81 ± 0.06

mg L^{-1} at Monroe Run to 3.51 ± 0.24 mg L^{-1} at Little Savage River. Percent methyl mercury of total mercury ranged from 9.43 % at Little Savage River to 14.71 % at Monroe Run.

Correlation Analyses

For Allegheny Mountain dusky salamanders both weight ($r = 0.262$, $P = 0.0065$) and SVL ($r = 0.369$, $P < 0.001$) were weakly but significantly correlated with ¼ root transformed total mercury concentrations. In northern two-lined salamanders and northern dusky salamanders weight, SVL, and total body length were not significantly correlated with ¼ root transformed total mercury concentrations (Table 15).

In only northern dusky salamanders stream total mercury ($r = 0.844$, $P = 0.0347$), stream methyl mercury ($r = 0.898$, $P = 0.0151$), and stream DOC ($r = 0.929$, $P = 0.0073$) were significantly correlated with ¼ root transformed tissue total mercury concentrations. In northern two-lined and Allegheny Mountain dusky salamanders no water chemistry factors were significantly correlated with ¼ root transformed tissue total mercury concentrations (Table 16). Despite non-significant *P* values, correlation coefficients were still high. In northern two-lined salamanders correlation coefficients for stream total mercury ($r = 0.646$, $P = 0.1657$), stream methyl mercury ($r = 0.776$, $P = 0.0696$), and stream DOC ($r = 0.702$, $P = 0.1199$) were slightly lower than those of the northern dusky salamanders. In Allegheny Mountain dusky salamanders the correlation coefficients for stream methyl mercury ($r = 0.748$, $P = 0.0874$) and stream DOC ($r = 0.673$, $P = 0.1430$) were slightly below those of the northern two-lined salamanders. Correlation analyses include data from all streams.

DISCUSSION

We hypothesized that northern two-lined salamanders would have higher concentrations of mercury than both northern dusky and Allegheny Mountain dusky salamanders. Our data indicates that northern two-lined salamanders do have significantly higher mercury concentrations than northern dusky salamanders at all streams and Allegheny Mountain dusky salamanders at most streams. Previous studies indicate northern two-lined salamanders accumulate significant amounts of mercury during the larval period (Banks et al. 2005, Castro et al. 2018). The longer larval period (2-3 years compared to 9-12 months), the aquatic diet of larval northern two-lined salamanders, and the placement of egg masses in the shallows of the stream may explain the higher total mercury concentrations found in the northern two-lined salamanders compared to northern dusky salamanders despite the aquatic diet of the adult northern dusky salamanders (Castro et al. 2018, Petranka 1998).

We also hypothesized that the Allegheny Mountain dusky salamander would have lower total mercury concentrations than the other two species due to the short to non-existent larval period and the more terrestrial diet of this species. Our results, however, indicate this was not the case. The lack of a significant difference in total mercury concentrations between the northern dusky salamanders and the Allegheny Mountain dusky salamanders does not support our hypothesis. At Mud Lick and Monroe Run, total mercury concentrations in Allegheny Mountain dusky and northern two-lined salamanders were similar and were greater than northern dusky salamanders found at the same streams (Fig. 5). This variation is not well explained by differences in stream chemistry as Mud Lick has significantly more total and methyl mercury and DOC than Monroe Run, though this variation may be explained by dietary variability between populations of Allegheny Mountain dusky salamanders. Sub-populations of Allegheny Mountain dusky salamanders found around different habitat types, such as seepages and stream edges, could have significantly different diets which could dramatically impact mercury accumulation between individuals captured from different habitats (Lannoo 2005, Petranka 1998). The work of Rimmer et al. (2010) indicates that terrestrial arthropods, the expected diet of the Allegheny Mountain dusky salamander (Lannoo 2005, Petranka 1998), are higher in mercury concentrations than we originally assumed. We removed one undigested caterpillar of an unknown species from the stomach of an Allegheny Mountain dusky salamander from Mill Run and analyzed it for total mercury. The caterpillar had a weight of 0.08 g and a total mercury concentration of 5.45 ng g^{-1}. Similar caterpillars were seen in the stomachs of other Allegheny Mountain dusky salamanders but were not analyzed due to more advanced digestion in those samples. This single sample was comparable to the low end of the range (7 ng g^{-1} to 108 ng $^{g-1}$) from *Lepidoptera* larvae published by Rimmer et al. Data from Castro et al. (2007) shows that this caterpillar has a lower concentration of total mercury than the mean concentrations measured in many species of aquatic macroinvertebrates collected from nearby streams. The large range published by Rimmer et al. is comparable to the data published by Castro et al. It is possible that the mercury concentrations found in the terrestrial arthropods being consumed by the Allegheny Mountain dusky salamander adults are comparable to the concentrations found the aquatic benthic macroinvertebrates being consumed by the northern dusky salamanders and the larvae of the northern two-lined salamanders. This would explain the statistically similar concentrations found between these species.

Differences in total mercury concentrations between species of stream salamander may also be explained by differences in metabolic efficiency. Northern dusky salamanders are significantly larger than the other two species (Table 12). It is possible that the lower concentrations of total mercury found in the northern dusky salamanders may be explained by a higher biomass dilution of the mercury. If northern dusky salamanders are more metabolically efficient they may be consuming and incorporating less mercury into their biomass, resulting in lower concentrations of total mercury in their tissues. The high concentrations of total mercury found in Allegheny Mountain dusky salamanders at Mud Lick and Monroe run may be explained by lower metabolic efficiency. If the Allegheny Mountain dusky salamanders are consuming a higher volume of prey per gram of biomass, they may be consuming and incorporating higher amounts of mercury. The same may be true of northern two-lined salamanders. Lower metabolic efficiency combined with the potentially high total mercury aquatic diet of the larval northern two-lined salamanders may explain the higher concentrations of total mercury. Studies of the metabolic rates of northern two-lined salamanders and Allegheny Mountain dusky salamanders indicate that these two species have similar metabolic energy assimilation efficiencies (~86%) (Fitzpatrick 1973, Fitzpatrick 1973). The metabolic energy assimilation of the northern dusky is unknown but other studies have indicated similar metabolic acclimation to temperature changes between northern dusky salamanders and Allegheny Mountain dusky salamanders (Fitzpatrick et al. 1971). Further study into the metabolic efficiencies of these species are needed to better understand the potential impacts this may have on the mercury concentrations in stream salamanders.

Only Allegheny Mountain dusky salamanders had tissue total mercury concentrations that were significantly correlated with weight and SVL, though these were weak correlations (weight: $r = 0.262$, SVL: $r = 0.369$). This may indicate that Allegheny Mountain dusky salamanders are accumulating mercury at similar rates throughout their lives and the other species have variable rates of accumulation between their larval and adult life stages.

Northern dusky salamanders were significantly correlated with stream total and methyl mercury and DOC. Total mercury concentrations in northern two-lined and Allegheny Mountain dusky salamanders were not significantly correlated with any water quality factors. Comparing correlation coefficients among the three species showed decreasing correlation between tissue total mercury and stream water chemistry factors from adult northern dusky to northern two-lined to Allegheny Mountain dusky salamanders. DOC concentrations in streams have been shown to affect mercury bioaccumulation in stream organisms (Balogh et al. 2003, Brigham et al. 2009). Based on the correlation coefficients between the three species, DOC is more positively correlated with tissue total mercury in the aquatic northern dusky salamanders than in the other two more terrestrial species. Life history accounts describe the northern dusky salamanders as staying closest to streams with northern two-lined salamanders staying close but venturing farther away, and the Allegheny Mountain dusky salamanders venturing the farthest from the streams. This indicates that as species become more terrestrial, total mercury concentrations are less correlated with stream water chemistry factors.

Mercury concentrations in all three species were highest at two streams, Little Savage River and Mud Lick. These results mirror the results of Castro et al. (2018), where the concentrations of adult and larval northern two-lined salamanders were significantly higher at these two streams than at other sampled streams. These two streams had the highest concentrations of both total and methyl mercury as well as DOC of the sampled streams. The higher stream concentrations of mercury may indicate higher concentrations of mercury in the prey species of the salamanders resulting in higher tissue concentrations in the adults. The high amount of variation in mercury concentration across streams may be indicative of dietary variability among different populations of the stream salamanders. Other researchers have observed dietary variability among different populations of many species of stream salamander, including the northern dusky, northern two-lined, and Allegheny Mountain dusky salamanders (Lannoo 2005, Petranka 1998).

CONCLUSION

Our results show that northern two-lined salamanders had significantly higher mercury concentrations than the other two species in this study. These results support our original hypothesis and we attribute this to the long larval stage of the northern two-lined salamanders. Contrary to our other hypothesis our results show that Allegheny Mountain dusky salamanders and northern dusky salamanders have statistically similar mercury concentrations. The higher than expected mercury concentrations in the

Allegheny Mountain dusky salamander may be a result of lower metabolic efficiencies in this species.

Dietary differences among the different species of stream salamander likely plays a large role in the mercury dynamics of these three species. Variability in diet both within a population and among different populations may also explain some of the variation in mercury concentrations seen in this study. Further study should focus on the potential microhabitat differences in mercury accumulation within populations of Allegheny Mountain dusky salamanders. It is likely that the terrestrial diet of the Allegheny Mountain dusky salamander is higher in mercury that was assumed at the outset of this study. Further studies should examine the dietary sources of mercury for salamanders and potential differences in metabolic efficiency among species, as this may drive a significant part of the variation in mercury concentrations measured across populations of salamanders. Study into the differences in mercury concentrations between the different life stages of northern dusky and Allegheny Mountain dusky salamanders can also increase the understanding of the dynamics behind mercury accumulation in stream salamanders.

TABLES AND FIGURES

Table 9 Streams sampled in Garrett County, Maryland, 2010 and number of salamanders collected per stream.

Stream Name	**Number of Salamanders**			**Salamanders Missing Tails**		
	ND	**NTL**	**MD**	**ND**	**NTL**	**MD**
Bear Pen	25	24	20	2	3	
Little Savage River	20	10	20	2		1
Mud Lick (ML)	30	30	6	1	1	
Monroe Run (MON)	30	16	21	5	1	2
Mill Run (MR)	29	20	20	4	1	2
Poplar Lick (PL)	30	11	20	1		
Total	164	111	107	15	6	5

Table 10 Watershed composition and land use of streams sampled in Garrett County, MD.

	Land Use (%)					Forest type (%)			Total Area
Stream	**Wet.**	**Ag.**	**Dev.**	**For.**	**Bare**	**Decid.**	**Ever.**	**Mixed**	**(Ha)**
Bear Pen	0	9.88	1.57	88.07	0.48	84.34	2.44	1.29	867
L. Sav. River	0.14	0.3	1.95	95.21	0.21	70.96	22.47	1.77	555
Mud Lick	0	29.1	7.73	61.92	1.2	42.92	18.07	0.93	1432
Monroe Run	0	2.68	4.8	92.46	0.05	87.69	3.97	0.8	1201
Mill Run	0	15.61	9.42	74.28	0.59	69.72	3.86	0.7	4365
Poplar Lick	0.18	5.46	3.36	89.74	0.9	67.68	19.81	2.26	2139

Table 11 Methods used in water chemistry analysis of streams in Garrett County, Maryland, 2010.

	Procedure for Sample Preparation	**Method of Analysis**
Dissolved Organic Carbon	EPA Method 415.3	Ultraviolet Absorbance at 254 nm
Total Suspended Solids	ESS Method 340.3	Filtration and Drying to 103-105 C
Major Anions (Cl^-, NO_3^-, SO_4^{2-})	EPA Method 300 Rev 2.1	Ion Chromatography
Acid Neutralizing Capacity		Gran Analysis Technique
pH	EPA Method 150.1	Electrometric Determination
Total Mercury	EPA Method 1631	Cold Vapor Atomic Fluorescence Spectrophotometry (CVAFS)
Methyl Mercury	EPA Method 1630	Ethylation and Preconcentration Purge and Trap Techniques

Table 12 Mean weight (±SE), SVL (±SE), and total length (±SE) for northern dusky (ND), northern two-lined (NTL), and Allegheny Mountain dusky salamanders (MD) sampled from Garrett County, MD. Individuals that had visibly lost a portion of their tail were not included in the average total length.

	Mean Weight (g)			Mean SVL (mm)			Mean Length (mm)		
Site	**ND**	**NTL**	**MD**	**ND**	**NTL**	**MD**	**ND**	**NTL**	**MD**
Bear Pen	2.22 ± 0.24	0.52 ± 0.05	0.90 ± 0.08	44.60 ± 1.66	30.78 ± 1.00	35.53 ± 1.14	86.83 ± 2.64	63.27 ± 2.62	73.63 ± 2.54
Little Savage River	2.08 ± 0.25	0.80 ± 0.12	0.89 ± 0.08	43.74 ± 1.78	35.57 ± 1.94	35.00 ± 1.37	83.17 ± 3.11	75.27 ± 4.89	73.39 ± 3.33
Mud Lick	2.04 ± 0.16	0.78 ± 0.06	1.02 ± 0.08	44.17 ± 1.35	35.55 ± 1.26	37.35 ± 1.29	88.61 ± 2.93	75.45 ± 3.28	77.20 ± 4.24
Monroe Run	3.17 ± 0.20	1.03 ± 0.10	1.06 ± 0.10	50.26 ± 1.06	38.48 ± 1.09	37.44 ± 1.36	96.99 ± 2.41	84.02 ± 3.33	77.18 ± 3.07
Mill Run	2.02 ± 0.19	0.84 ± 0.10	0.94 ± 0.13	44.64 ± 1.55	35.53 ± 1.69	34.34 ± 2.12	88.00 ± 3.16	80.71 ± 4.68	75.31 ± 5.37
Poplar Lick	2.23 ± 0.19	0.93 ± 0.14	1.15 ± 0.09	45.28 ± 1.50	35.74 ± 1.97	37.29 ± 1.29	88.97 ± 3.07	78.85 ± 5.29	80.17 ± 3.19

Table 13 Mean total mercury concentrations (□SE) for adult northern dusky (ND) Allegheny Mountain dusky (MD) and northern two-lined (NTL) salamanders in streams in Garrett county, Maryland, 2010. Asterisks indicate mercury concentrations at Little Savage River and Mud Lick were significantly higher for all three species.

	Mean Total Mercury (ng g^{-1})					
Stream	**ND**	**n**	**NTL**	**n**	**MD**	**n**
Bear Pen	14.59 ± 1.09	25	24.54 ± 1.65	24	15.66 ± 1.16	20
Little Savage River	32.08 ± 2.40 *	20	36.53 ± 4.34 *	10	32.75 ± 3.69 *	20
Mud Lick	28.66 ± 2.06 *	30	40.97 ± 2.69 *	30	44.20 ± 5.55 *	6
Monroe Run	16.26 ± 0.84	30	22.87 ± 1.56	16	23.40 ± 2.14	21
Mill Run	17.10 ± 1.17	29	19.36 ± 1.76	20	15.84 ± 1.43	20
Poplar Lick	19.55 ± 1.32	30	31.45 ± 4.64	11	20.14 ± 1.61	20
Overall Mean	20.95±0.78	164	29.57±1.32	111	22.84±1.23	107

Table 14 Mean dissolved organic carbon (DOC) (±SE), total mercury (THg) (±SE), methyl mercury (MeHg) (±SE), and methylation efficiency (MeHgeff) in streams in Garrett County, MD.

Stream	Mean DOC mg L^{-1}	Mean THg ng L^{-1}	Mean MeHg ng L^{-1}	Mean MeHg Percent
Bear Pen	0.84 ± 0.06	0.66 ± 0.05	0.09 ± 0.02	12.59
Little Savage River	3.51 ± 0.24	2.25 ± 0.21	0.21 ± 0.05	9.43
Mud Lick	2.06 ± 0.25	1.00 ± 0.10	0.15 ± 0.03	14.56
Monroe Run	0.81 ± 0.06	0.60 ± 0.06	0.09 ± 0.02	14.71
Mill Run	1.02 ± 0.07	0.52 ± 0.06	0.06 ± 0.01	12.26
Poplar Lick	0.95 ± 0.06	0.76 ± 0.09	0.08 ± 0.02	10.74
Overall Mean	1.39 ± 0.14	0.94 ± 0.08	0.11 ± 0.01	12.06 ± 0.88

Table 15 Pearson product-moment correlation tables of ¼ root transformed total mercury concentrations in adult northern dusky (ND) allegheny mountain dusky (MD) and northern two-lined (NTL) salamanders and salamander weight, snout-vent length (SVL), and total length. Asterisks indicate significant correlations (*P* < 0.05).

	RootTotal Hg	Weight	SVL
ND			
RootTotal Hg			
Weight	0.059		
SVL	0.126	0.940*	
Length	-0.063	-0.385*	-0.293*
NTL			
RootTotal Hg			
Weight	0.098		
SVL	0.143	0.947*	
Length	-0.023	0.229*	0.291*
MD			
RootTotal Hg			
Weight	0.262*		
SVL	0.369*	0.921*	
Length	0.135	0.578*	0.627*

Table 16 Pearson product-moment correlation coefficients for ¼ root transformed total mercury concentrations in adult northern dusky (ND) Allegheny Mountain dusky (MD) and northern two-lined (NTL) salamanders and stream water chemistry factors. Asterisks indicate significant correlations ($P < 0.05$).

	RootTotal Hg ND	**RootTotal Hg NTL**	**RootTotal Hg MD**
pH	-0.701	-0.572	-0.397
ANC	-0.26	-0.26	0.05
THg	0.844*	0.646	0.537
MeHg	0.898*	0.776	0.748
DOC	0.929*	0.702	0.673
TSS	0.14	0.378	-0.093
Cl	0.077	-0.146	0.177
NO3	-0.671	-0.566	-0.375
SO4	-0.428	-0.696	-0.567

Figure 4 Stream Sites sampled in the Savage River and Youghiogheny River Watersheds

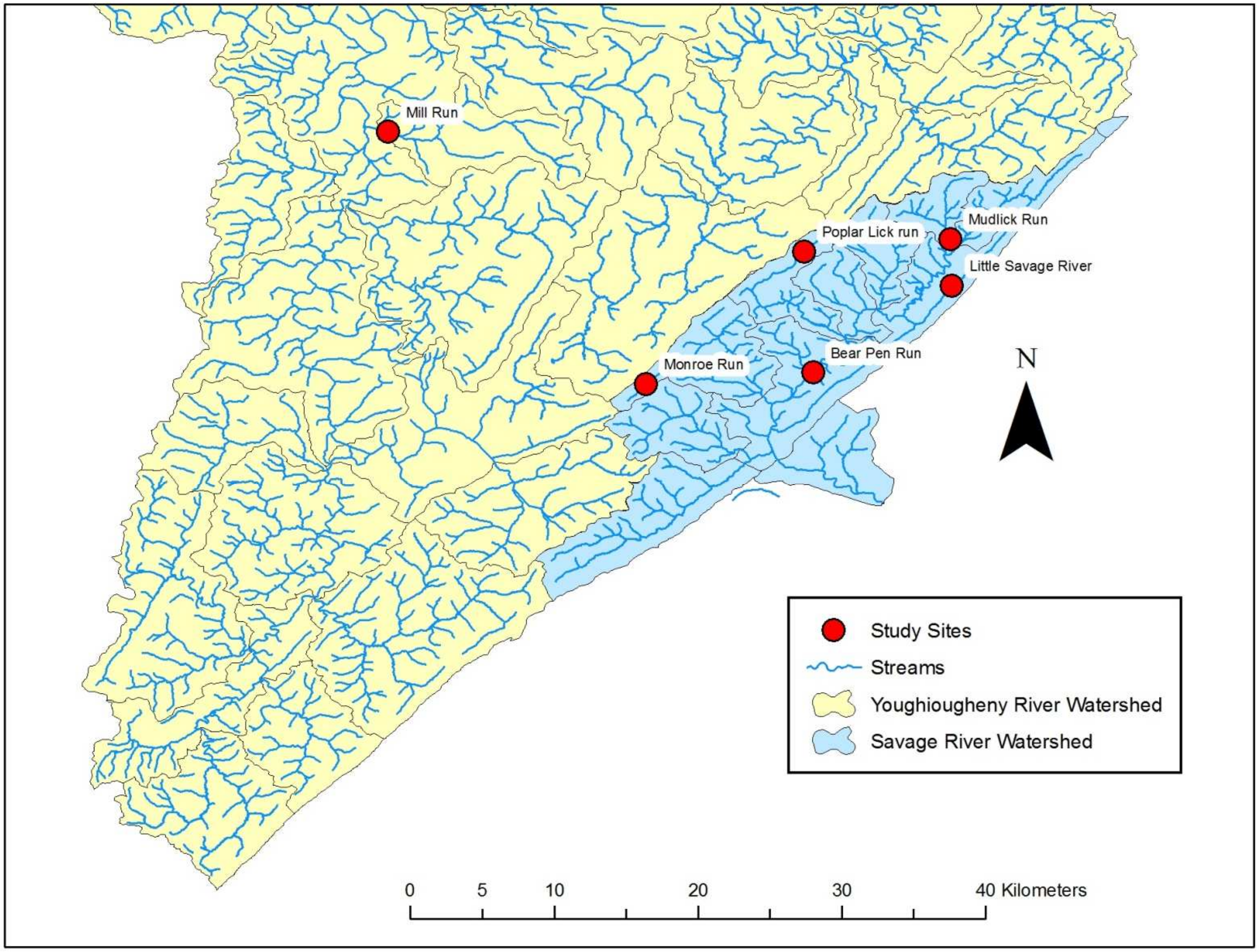
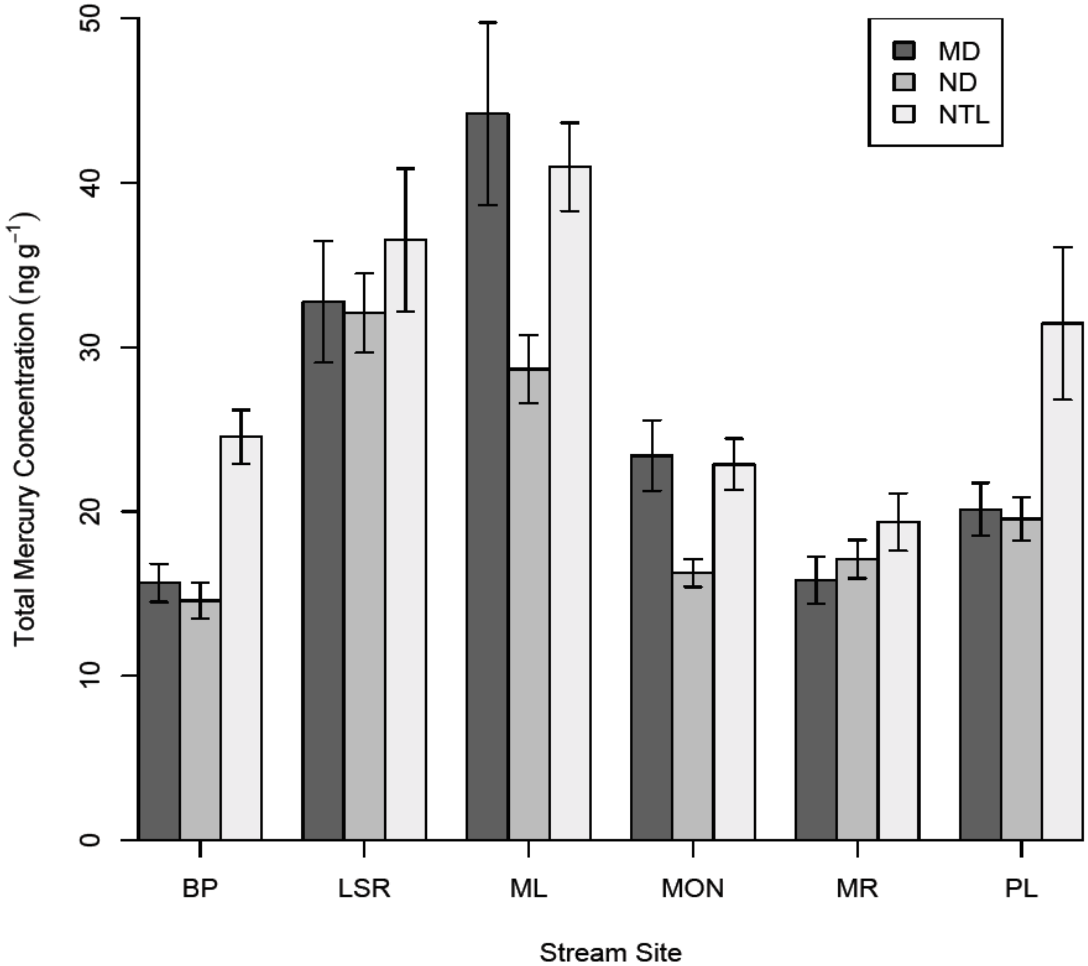


Figure 5 Mean total mercury concentrations (ng g-1) for adult northern dusky (ND) Allegheny Mountain dusky (MD) and northern two-lined (NTL) salamanders for each sampled stream, Bear Pen (BP), Little Savage River (LSR), Mud Lick (ML), Monroe Run (MON), Mill Run (MR), and Poplar Lick (PL). Error bars are ± 1 SE.



LITERATURE CITED

Balogh SJ, Yabing H, Offerman H, Meyer M, Johnson D. Methylmercury in rivers draining cultivated watersheds. Sci Total Environ 2003; 304: 305-313.

Bank MS, Loftin C, Jung R. Mercury Bioaccumulation in Northern Two-Lined Salamanders from Streams in the Northeastern United States. Ecotoxicology 2005; 14: 181-191.

Ben-Ari E. A New Piece in the Puzzle of Global Amphibian Declines. Bioscience 2005; 55, 1: 96.

Bergeron CM, Bodinof C, Unrine J, Hopkins W. Mercury Accumulation Along a Contamination Gradient and Nondestructive Indices of Bioaccumulation in Amphibians. Environ Toxicol Chem 2010; 29, 4: 980-988.

Boone MD, James S. Interactions of an Insecticide, Herbicide, and Natural Stressors in Amphibian Community Mesocosms. Ecol Appl 2003; 13, 3: 829-841.

Brigham ME, Wentz D, Aiken G, Krabbenhoft D. Mercury Cycling in Stream Ecosystems. 1. Water Column Chemistry and Transport. Environ Sci Technol 2009; 43,8: 2720-2725.

Brooks S, Moore C, Lew D, Lefer B, Huey G, Tanner D. Temperature and sunlight controls of mercury oxidation and deposition atop the Greenland ice sheet. Atmos Chem Phys Discus 2011; 11: 3663-3691.

Burke JN, Bergeron C, Todd B, Hopkins W. Effects of mercury on behavior and performance of northern two-lined salamanders (Eurycea bislineata). Environ Pollut 2010; 158: 3546-3551.

Castro MS, Hilderbrand R, Thompson J, Heft A, Rivers S. Relationships Between Wetlands and Mercury in Brook Trout. Arch Environ Contam Toxicol 2007; 52: 97-103.

Castro, MS, Hilderbrand, RH, Kaumeyer M. Mercury Concentrations in Northern Two-Lined Salamanders from Stream Ecosystems in Garrett County, Maryland. Arch Environ Contam and Toxicol 2018; 75: 17-24.

Cremona F, Planas D, Lucotte M. Assessing the importance of macroinvertebrate trophic dead ends in the lower transfer of methylmercury in littoral food webs. Can J Fish Aquat Sci 2008; 65, 9: 2043-2052.

Driscoll CT, Han Y, Chen C, Evers D, Lambert K, Holsen T, Kamman N, Munson R. Mercury Contamination in Forest and Freshwater Ecosystems in the Northeastern United States. Bioscience 2007; 57, 1: 17-28.

Fitzgerald WF, Engstrom DR, Mason RP, Nater EA. The case for atmospheric mercury contamination in remote areas. Environ Sci Technol 1998; 32:1–7.

Fitzpatrick LC, Bristol JR, Stokes RM. Thermal Acclimation and Metabolic Rates in the Dusky Salamander, *Desmognathus fuscus*. Comp Biochem Physiol 1972; 41a: 89-96.

Fitzpatrick LC. Energy Allocation in the Allegheny Mountain Salamander, Desmognathus Ochrophaeus. Ecol Monogr 1973; 43,1: 43-58.

Fitzpatrick LC. Influence of Seasonal Temperatures on the Energy Budget and Metabolic Rates of the Northern Two-Lined Salamander, *Eurycea bislineata bislineata*. Comp Biochem Physiol 1973; 45, 3: 807-818.

Gilmour CG and Henry EA. Mercury Methylation in Aquatic Systems Affected by Acid Deposition. Environ Pollut 1991; 71: 131–69.

Hatch AC, Blaustein A. Combined Effects of UV-B Radiation and Nitrate Fertilizer on Larval Amphibians. Ecol Appl 2003; 13, 4: 1083-1093.

Kelly EN, Schindler D, St. Louis V, Donald D, Vladicka K. Forest Fire Increases Mercury Accumulation by Fishes via Food Web Restructuring and Increased Mercury Inputs. Proc Nat Acad Sci USA 2006; 103, 51: 19380-19385.

Lanoo M. Amphibian Declines: The Conservation Status of United States Species. Berkeley and Los Angeles, CA: University of California Press; 2005.

Lenkowski JR, Reed J, Deininger L, McLaughlin K. Perturbation of Organogenesis by the Herbicide Atrazine in the Amphibian Xenopus laevis. Environ Health Perspect 2008; 116, 2: 223-230.

Mason RP, Laporte JM, Andres S. Factors Controlling the Bioaccumulation of Mercury, Methylmercury, Arsenic, Selenium, and cadmium by Freshwater Invertebrates and Fish. Arch Environ Contam Toxicol 2000; 38: 283-297.

Mendelson III JR. Confronting Amphibian Declines and Extinctions. Science 2006; 313, 5783: 48.

Moore CW, Cosby B, Galloway J, Castro M. Relationship Between pH and Stream Water Total Mercury Concentrations in Shenandoah National Park. Water Air Soil Pollut 2009; 201: 233-238.

Morel FM, Kraepiel A, Amyot M. The Chemical Cycle and Bioaccumulation of Mercury. Annu Rev Ecol Syst 1998; 29: 543-566.

National Atmospheric Deposition Program / Mercury Deposition Network (NADP/MDN). (2004). NADP Program Office, Illinois State Water Survey, 2204 Griffith Drive, Champaign, IL 61820, http://nadp.sws.uiuc.edu/.

Peckenham JM, Kahl J, Nelson S, Johnson K, Haines T. Landscape Controls on Mercury in Streamwater at Acadia National Park, USA. Environ Monit Assess 2007; 126: 97-104.

Petranka JW. Ontogeny of the Diet and Feeding Behavior of *Eurycea bislineata* Larvae. J Herpetol 1984; 8, 1: 48-55.

Petranka JW. Salamanders of the United States and Canada. Washington D.C. Smithsonian Institution Press; 1998.

Jose Pinheiro, Douglas Bates, Saikat DebRoy, Deepayan Sarkar and the R Development Core Team (2011). nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-98.

Prepas EE, Gabos S, Strachan W, Weiping Z. Methyl mercury concentrations in macroinvertebrates and fish from burned and undisturbed lakes on the Boreal Plain. Can J Fish Aquat Sci 2005; 62, 9: 1963-1977.

R Development Core Team (2009). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Rimmer CC, Miller EK, McFarland KP, Taylor RJ, Faccio FD. Mercury bioaccumulation and trophic transfer in the terrestrial food web of a montane forest. Ecotoxicol 2010; 19: 697-709.

Rouse JD, Bishop C, Struger J. Nitrogen Pollution: An Assessment of Its Threat to Amphibian Survival. Environ Health Perspect 1999; 107, 10: 799-803.

Rovito SM, Parra-Olea G, Vasquez-Almazan C, Papenfuss T, Wake D. Dramatic declines in neotropical salamander populations are an important part of the global amphibian crisis. Proc Nat Acad Sci USA 2009; 106: 3231-3236.

Schiesari L, Grillitsch B, Grillitsch H. Biogeographic Biases in Research and Their Consequences for Linking Amphibian Declines to Pollution. Conserv Biol 2007; 21, 2:465-471.

Shanley JB, Kamman N, Clair T, Chalmers A. Physical Controls on Total and Methylmercury Concentrations in Streams and Lakes of the Northeastern USA. Ecotoxicol 2005; 14: 125-134.

Southerland MT, Jung R, Baxter D, Chellman I, Mercurio G, Volstad J. Stream Salamanders as Indicators of Stream Quality in Maryland, USA. Appl Herpetol 2004; 2: 23-46.

Stokstad E. Global Survey Documents Puzzling Decline of Amphibians. Science 2004; 306, 5695: 391.

Storrs SI, Kiesecker J. Survivorship Patterns of Larval Amphibians Exposed to Low Concentrations of Atrazine. Environ Health Perspect 2004; 112, 10:1054-1057.

Taylor B, Skelly D, Demarchis L, Slade M, Galusha D, Rabinowitz P. Proximity to Pollution Sources and Risk of Amphibian Limb Malformation. Environ Health Perspect 2005; 113, 11: 1497-1501.

Wolfe MF, Schwarzbach S, Sulaiman RA. Effects of Mercury on Wildlife: A Comprehensive Review. Environ Toxicol Chem 1998; 17: 146–160.

Zizek S, Horvat M, Gibicar D, Fajon V, Toman M. Bioaccumulation of mercury in benthic communities of a river ecosystem affected by mercury mining. Sci Total Environ 2007; 377, 2: 407-415.

CHAPTER 3: THE INFLUENCE OF WATER CHEMISTRY FACTORS ON TISSUE TOTAL MERCURY CONCENTRATIONS IN STREAM SALAMANDERS IN GARRETT COUNTY MARYLAND

ABSTRACT

This study examined the potential influence of stream water chemistry on tissue total mercury concentrations in three species of salamander in Garrett County, MD. We collected adults of two species, the northern two-lined salamander (*Eurycea bislineata bislineata*) and the northern dusky salamander (*Desmognathus fuscus*) in April, July, and September 2010. Northern two-lined salamanders were collected from seven first order streams, and northern dusky salamanders were collected from 6 first order streams. Adult Allegheny Mountain dusky salamanders (*Desmognathus ocrophaeus*) were collected in July and September 2010 from nine first order streams. Water chemistry was sampled monthly from April 2010 through December 2010, excluding September. Mean salamander tissue total mercury concentrations were significantly correlated with mean stream methyl mercury concentrations in northern dusky salamanders (P = 0.0180, r = 0.888), and Allegheny Mountain dusky salamanders (P = 0.0361, r = 0.699). Regression analysis indicated that stream total mercury, dissolved organic carbon (DOC), and stream sulfate concentrations were significant drivers of stream methyl mercury concentrations. Mean stream DOC was significantly correlated with mean salamander tissue total mercury concentrations in Allegheny Mountain dusky salamanders (P = 0.0268, r = 0.726) and northern dusky salamanders (P = 0.0053, r = 0.940). Regression analysis

indicated a significant relationship between DOC and stream total mercury, stream chloride, and stream nitrate. Stream water chemistry has a significant influence on salamander mercury concentrations, specifically stream mercury concentrations and DOC, though further study is needed to better understand these relationships.

INTRODUCTION

The volatile chemical nature of mercury and the variety of anthropogenic sources of mercury to the atmosphere, such as the emissions of coal fired power plants, have led to the global distribution of mercury pollution. The mercury biogeochemical cycle is complex, involving chemical transformations in the atmosphere and chemical and biological transformations in terrestrial and aquatic ecosystems. In the atmosphere, elemental mercury (Hg^0) is transformed to the more reactive form (Hg^{2+}), which then forms complexes with a variety of halogens and can be rapidly deposited to the landscape (Lindberg et al. 2007). There, mercury is converted to methyl mercury through the actions of sulfate-reducing bacteria and other bacteria species in moist, anaerobic soils and under anaerobic conditions in wetland and aquatic environments (Langley 1973, Gilmour et al. 1991). Methyl mercury is more toxic and bio available than the elemental form of mercury. This toxic form can be readily accumulated by organisms and magnified up the trophic scale (Mason et al. 1996, Ward et al. 2010).

The drivers and mechanisms of mercury methylation have been studied extensively over the past several decades (Langley 1973, Shin and Krenkel 1976, Morel et al. 1998, Driscoll et al. 2007). However, the drivers and mechanisms of the movement of mercury through food chains are less understood. Mercury bioaccumulation has been studied extensively in fish (Gilmour and Riedel 2000, Swanson et al. 2003, Hogan et al. 2007). There is little information about mercury bioaccumulation in stream salamanders. In addition, there has been little study on the influence of stream water chemistry on salamander tissue total mercury concentrations.

Dissolved organic carbon (DOC) and total suspended solids (TSS) in stream water have been identified as important transport vectors of mercury through aquatic environments (Grigal 2002). DOC and TSS mobilize mercury allowing for uptake by producers and eventually consumers up the food web.

The importance of sulfate-reducing bacteria in the methylation of mercury also indicates the potential for sulfate concentrations in streams to be an important driver of stream mercury concentrations and the bioaccumulation of mercury. Many stream and lake-based studies have identified a strong positive correlation between sulfate additions to lakes and streams and increasing mercury methylation (Gilmour et al. 1992).

Potentially related to this relationship is stream pH and acid neutralizing capacity (ANC); lower pH has been linked to increased mercury concentrations in stream ecosystems. ANC is strongly linked to stream pH. As stream ANC decreases the stream water is less able to buffer acidic inputs and the pH of the stream is more likely to dramatically decrease during precipitation events due to the acidic nature of rainfall. Acidification of stream waters can lead to increased availability of metals, including mercury, in the stream water (Gilmour et al. 1992, Ward et al. 2010). Increased availability of mercury may increase bioaccumulation in stream organisms.

Stream ecosystems are strongly linked to the surrounding watershed. Not only is water chemistry directly tied to the geology of the surrounding region, it is also strongly influenced by the plant community found around the stream. Streams found in evergreen forests often have very different water chemistry than those found in deciduous forests or in grasslands. Stream food webs are also strongly linked with the food web in the surrounding watershed. Species such as salamanders, with aquatic and terrestrial life stages, act as a direct link between the two food webs (Petranka 1998, Lanoo 2005). For this reason, salamanders may play an important and little-studied roll in the movement of mercury throughout the landscape (Bank et al. 2005). The terrestrial adults of many stream salamander species roam well into riparian zones carrying mercury accumulated from the stream during their aquatic larval stage and are important food items for many forest inhabitants including birds, snakes, raccoons, foxes, and other predators. Previous studies have shown significant concentrations of mercury in stream salamanders (Bank et al. 2005, Bergeron et al. 2010, Burke et al. 2010).

The purpose of this project was to determine the influence of stream water chemistry on tissue total mercury concentrations in stream salamanders. We measured mercury in three species of salamander, the northern dusky salamander (*Desmognathus fuscus*), the northern two-lined salamander (*Eurycea bislineata bislineata*), and the Allegheny Mountain dusky salamander (*Desmognathus ocraphaeus*) from streams in Garrett County, MD. We simultaneously sampled stream water for pH, ANC, total mercury concentration, methyl mercury concentration, DOC, TSS, chloride concentration, nitrate concentration, and sulfate concentration. Our goal was to correlate these water chemistry variables with variations in total mercury concentration in salamander tissues.

METHODS

Stream Selection and Study Sites

Our study streams were located in Garrett County, Maryland and were in the Savage River and Youghiogheny River watersheds (Fig. 6). Streams were first order and drained watersheds of similar land use. Watersheds ranged from 58 to 96 percent forest, and <1 to 32 % agriculture. Watershed area ranged from 1543 acres to 10929 acres at Mill Run. The largest watershed, Mill Run, is twice the size of the next larger watershed in the study, Poplar Lick (Table 18).

Field Sampling

We collected salamanders in April, July, and September of 2010 using visual encounter surveys by overturning cover objects within 2 m of the stream edge. Survey sampling for salamanders lasted for 2-6 hours at each site. Salamanders were collected using a small aquarium dip net and were transported in glass jars to the Appalachian Lab in Frostburg, MD. We attempted to collect 10 adults per site for each sampling. The actual number of samples varied due to time, weather, and other sampling constraints (Table 17).

Water Chemistry

Stream water was sampled monthly from April through December 2010 with the exception of September. Measurements of water temperature, specific conductivity, and pH were made *in situ* using a Hydrolab Quanta model sonde. Sonde pH and conductivity sensors were calibrated prior to all sampling trips. Grab samples of stream water were collected with zero headspace in acid cleaned high density polyethylene (HDPE) bottles for laboratory analysis of closed pH, ANC, total and methyl mercury, dissolved organic carbon (DOC), total suspended solids (TSS), chloride (Cl^{-1}), nitrate (NO_3^{-1}), and sulfate

(SO_4^{-2}). Water samples were filtered using Whatman grade 43 16 μm glass fiber filters in the lab for TSS and DOC.

Total Mercury in Salamanders

Salamanders were euthanized in a buffered 10 g/L solution of MS-222. Once euthanized, salamanders were measured for weight, total length, and snout-vent length (SVL). Salamanders were then frozen in a -22ºC freezer and until analysis. All analyses were completed within 3 months of sample collection. The entire salamander (.05 – 5 g) was digested in 70% concentrated sulfuric acid and 30% concentrated nitric acid. We diluted 100-150 μL of digestion solution to 50 mL with distilled deionized water.

The total mercury concentration in the digests was measured by CVAFS on a Tekran 2600 CVAFS mercury analysis system (EPA 1631). Instrument calibration required an $r^2 > 0.99$ for the calibration curve. All digests were diluted to fit within our standard operating range (0.2 – 56.6 ng/g). Duplicate analysis of digests was done on every tenth tissue digest, but low sample weights precluded replicate analysis of individual salamanders. Duplicates were acceptable with < 5% difference between the replicates. DORM-3 fish protein (National Research Council Canada) was used a certified reference material. Averaged recovery of DORM-3 samples carried through the digestion and analysis procedure was 368 ng/g. The acceptable range of mercury concentrations for the DORM-3 samples is 322 to 442 ng/g. Digest blanks served as analytical blanks. Digest blanks diluted and analyzed in the same way as samples 0.7 ± 0.3 ng/L.

Total Mercury and Methyl Mercury in Stream Water

Mercury samples were collected in ultra clean Teflon bottles and double Ziploc bagged using the "clean hands/dirty hands" technique (EPA method 1639). All samples were placed on ice in the field and transported in coolers to the Appalachian Environmental Laboratory (AEL) for analysis. THg and MeHg samples were stored at -4ºC and were analyzed within 1 month of collection. THg and MeHg samples were unfiltered prior to analysis due to low TSS concentrations. Prior to analysis concentrated bromine monochloride was added to each THg sample. MeHg samples were acidified using concentrated hydrochloric acid prior to analysis. Ethylation of these samples was performed using sodium tetraethyl borate. Analyses were performed using the appropriate EPA standard analysis technique (Table 19). Field replicates were randomly collected from three streams for each monthly water chemistry sampling, and 2 lab duplicates were analyzed for every set of samples.

Statistical Analysis

Water chemistry data was transformed using a $\log_{10}$ transformation prior to analysis, and salamander tissue total mercury data was transformed using a ¼ root transformation. The mean was then calculated for each water chemistry parameter at each site. These means were then run in a Pearson Product-Moment Correlation analysis with the mean tissue mercury concentrations for each species at each site. Water chemistry factors that were significantly correlated with salamander tissue total mercury concentrations were used as response variables in a multiple regression analysis. Models were constructed using a backwards elimination approach resulting in all predictive variables significantly related to the response variables and the lowest Bayesian information criteria (BIC). Square root transformations were used to meet the homogeneity of variances and normality of residuals assumptions of multiple regression analyses. All statistical analyses were performed using the R-Project (version 2.12) (R Development Core Team 2009).

RESULTS

Water Chemistry

The first-order Appalachian streams sampled in this study were circumneutral, ionically dilute and low in DOC and suspended solids. Nitrate averaged about 0.5 mg/L and sulfate about 10 mg/L. Exceptions were Murley Run, where stream water pH was usually below 5, and ANC was often negative.

Murley Run and Little Savage River had pH values below those of the other streams, 5.98 ± 0.10 at Little Savage River and 4.59 ± 0.04 at Murley Run.

ANC varied across streams. Most streams had ANC values above 100 μeq/L, the two exceptions were again Little Savage River (43.9 ± 12.3 μeq/L) and Murley Run (-22.3 ± 2.3 μeq/L).

Laurel Run experienced a large swing in pH and ANC during the sampling period. Crushed limestone was added upstream of our sampling site in an effort to remediate low pH between the May and June samplings. This addition resulted in an increase of pH from 4.7 to 7.2 and an increase in ANC from -8.56 μeq/L to 1198.97 μeq/L (Fig. 10).

Streams fall into two categories based on total mercury concentrations. Four streams had mean total mercury concentrations 1.00 ng/L or greater and five streams had total mercury concentrations below 1.00 ng/L. The highest mean total mercury concentration was measured at Little Savage River (2.25 ± 0.21 ng/L).

Mean methyl mercury concentration exhibited similar variation to total mercury. Three streams had mean methyl mercury concentrations above 0.10 ng/L, with the highest concentration measured at Little Savage River (0.21 ± 0.05 ng/L). The mean methyl mercury concentrations at the other six streams ranged from 0.03 ± 0.00 ng/L to 0.09 ± 0.02 ng/L. The percentage of total mercury present as methyl mercury ranged from 3.20 ± 2.4 % at Laurel Run to 14.82 ± 8.9 % at Murley Run.

There was not wide variation in DOC or TSS across streams. Five streams had mean DOC concentrations above 1.00 mg/L with the highest mean concentration of DOC at Little Savage River (3.51 ± 0.24 mg/L). Four streams had mean DOC concentrations below 1.00 mg/L. Mean TSS concentrations ranged from 1.17 ± 0.45 mg/L at Monroe Run to 2.76 ± 0.68 mg/L at Bear Pen.

Chloride and Sulfate showed wide variation across sites while nitrate showed little variation. Nitrate concentrations in all streams were less than 0.60 mg/L. Mill Run had the highest mean concentration of 0.55 ± 0.11 mg/L. Mill Run (90.43 ± 19.1 mg/L) and Mud Lick (74.78 ± 20.1 mg/L) have the highest chloride concentrations. These two sites experience large increases in chloride concentrations during the fall and winter months (Fig. 10). The lowest mean chloride concentration was 0.56 ± 0.03 mg/L at Murley Run. The streams with the highest sulfate concentrations were Mill Run (18.44 ± 1.64 mg/L) and Laurel Run (17.06 ± 1.43 mg/L). The streams with the lowest concentrations were Poplar Lick (8.02 ± 0.58 mg/L) and Little Savage River (8.05 ± 0.78 mg/L).

Mercury in Salamanders

The overall mean tissue total mercury concentration for northern dusky salamanders was 20.95 ± 0.78 ng/g within a range of 7.47 ng/g to 59.98 ng/g. Across individual streams mean tissue total mercury ranged from 14.59 ± 1.09 ng/g at Bear Pen to 32.08 ± 2.40 ng/g at Little Savage River.

The overall mean tissue total mercury concentration for northern two-lined salamanders was 29.54 ± 1.30 ng/g within a range of 10.92 ng/g to 73.78 ng/g. Across individual streams mean tissue total mercury concentrations ranged from 19.36 ± 1.76 at Mill Run to 40.97 ± 2.69 at Mud Lick.

The overall mean tissue total mercury concentration for Allegheny Mountain dusky salamanders was 22.84 ± 1.23 ng/g within a range of 6.26 ng/g to 77.53 ng/g. Across individual sites tissue total mercury concentrations ranged from 15.66 ± 1.16 ng/g at Bear Pen to 44.20 ± 5.55 ng/g at Mud Lick.

Correlation Analyses

Mean salamander tissue total mercury concentrations were significantly correlated with mean stream methyl mercury concentrations in northern dusky salamanders (P = 0.018, r = 0.888) and Allegheny Mountain dusky salamanders (P = 0.0361, r = 0.699). Mean stream DOC was significantly correlated with mean salamander tissue total mercury concentrations in Allegheny Mountain dusky salamanders (P = 0.0268, r = 0.726) and northern dusky salamanders (P = 0.0053, r = 0.940). Mean stream total mercury concentrations were significantly correlated with mean salamander tissue total mercury concentrations in northern dusky salamanders (P = 0.0352, r = 0.843). Stream sulfate concentrations were significantly correlated with tissue total mercury in Northern Two Lined Salamanders (P = 0.0396, r = 0.865).

Regression Analyses

Square root transformed stream methyl mercury concentration was best predicted by a model including stream total mercury concentration, stream DOC, and stream SO_4^{-2} concentration ($P < 0.001$, $r^2 = 0.454$).

(Eq. 1) $$\mathbf{[MeHg] = (0.045[THg] + 0.041[DOC] - 0.010[SO_4^{-2}] + 0.304)^2}$$

Negative square root transformed stream DOC was best predicted by a model including stream total mercury concentration, stream chloride and nitrate concentrations ($P < 0.001$, $r^2 = 0.429$).

(Eq. 2) $$\mathbf{[DOC] = (-0.194*[THg] - 0.002*[Cl^{-1}] + 0.308*[NO_3^{-1}] + 1.125)^{-2}}$$

DISCUSSION

Mercury in Salamanders

There was considerable variation in total mercury concentrations among the three species of salamander and across the study streams (Table 22). These concentrations are comparable to mercury concentrations measured in northern two-lined larvae in Acadia National Park (47.7 ± 1.9 ng/g to 79.5 ± 5.2 ng/g) and Shenandoah National Park (26.8 ± 1.6 ng/g), though they are lower than those measured at reference sites along the South

River in Virginia (256 ± 56 ng/g) (Bank et al. 2005, Bergeron et al. 2010, Burke et al. 2010).

Stream Methyl Mercury

Two of the three species of stream salamander we collected had mean tissue total mercury concentrations that were significantly correlated with stream methyl mercury concentrations (Table 21). This significant correlation illustrates the link between the stream salamanders and the aquatic food web where methyl mercury is magnified up increasing trophic levels. Northern dusky salamanders have the most aquatic adult life stage of the three species, northern two lined salamanders have the longest aquatic larval stage, but a more terrestrial adult stage, and Allegheny Mountain dusky salamanders are mostly terrestrial (Petranka 1998, Lannoo 2005). The correlation coefficients of salamander tissue total mercury and stream methyl mercury decrease as the adult stage of the salamanders becomes more terrestrial. While not significant in northern two lined salamanders, the correlation coefficient ($P = 0.0621$, $r = 0.731$) falls between that of the northern dusky salamanders ($r = 0.888$) and the Allegheny Mountain dusky salamander ($r = 0.699$). This indicates that the more terrestrial adults, northern two-lined and Allegheny Mountain dusky salamanders, may incorporate mercury sources of a terrestrial nature, though the majority of mercury is still likely incorporated from aquatic sources.

The backwards elimination method used to create a regression model to predict stream methyl mercury concentration resulted in a model consisting of stream total mercury concentration, stream DOC, and stream sulfate concentration (Eq. 1). This model matches well with literature descriptions of stream mercury methylation (Gilmour et al. 1991, Gilmour et al. 1992, Benoit et al. 2003, Ward et al. 2010). The positive coefficient for stream total mercury is indicative of the within-stream methylation that accounts for most of the methyl mercury in the stream water (Gilmour et al. 1991). Increasing total mercury concentrations indicates increased inorganic mercury available for methylation within the stream.

The positive influence of DOC on methyl mercury illustrates a potential transport relationship between DOC and mercury in the streams; previous studies have shown DOC to be an important transport vector for both inorganic and methyl mercury (Grigal 2002, Wu et al. 2004, Miller et al. 2007, Brigham et al. 2009). Increasing DOC in the streams potentially mobilizes more total and methyl mercury resulting in increased concentrations of methyl mercury in the stream water.

Previous studies involving sulfate additions to aquatic systems have shown an increase in mercury methylation after those additions (Gilmour et al. 1992). The negative regression coefficient for sulfate concentration in our model seems contradictory to past experimental evidence. This can be explained by the activity of sulfate-reducing bacteria. In the absence of a sulfate source, increased activity by sulfate-reducing bacteria may result in both a decrease in sulfate concentration as well as an increase in methyl mercury concentration.

This is best illustrated at our Murley Run site. Murley Run has the highest sulfate concentrations of any of our sampled streams and time series plots of sulfate concentration and methyl mercury concentration show strong correlation between the two ($r^2 = 0.794$, $n = 8$) (Fig. 7, 8). This highly significant relationship at Murley Run explains the significance of sulfate concentration in our regression model; the relationship between sulfate concentration and methyl mercury concentration is only significant at Murley Run (P = 0.003, r^2 = 0.794) and Monroe Run (P = 0.0496, r^2 = 0.418). The lack of a significant relationship at the other sites may be explained by the proximity of sampling locations at Murley Run and Monroe Run to wetland areas. Wetland areas have been shown to be important sites of mercury methylation (Castro et al. 2006). The sampling site at Murley Run was within 500 m of an inundated wetland region, which could be contributing to this significant relationship. The sampling site at Monroe Run was close to a wide, flat floodplain that is often saturated. This floodplain may be acting as a methylation site similar to a wetland. Mud Lick also has a similar floodplain to Monroe Run, and despite sulfate concentrations not being significantly related to methyl mercury concentrations, the regression coefficient at this site is the highest of the other sites (P = 0.161, r^2 = 0.181).

Stream DOC

Stream DOC was significantly correlated with tissue total mercury concentrations in northern dusky salamanders and Allegheny Mountain dusky salamanders. This may be explained by the role of DOC in complexing with inorganic and methyl mercury in the streams (Grigal 2002, Wu et al. 2004, Miller et al. 2007, Brigham et al. 2009). Most of our streams had low DOC concentrations, which may indicate low watershed inputs of DOC.

The regression model for DOC was also generated using the backward elimination method. Our resulting model consisted of stream total mercury concentration, stream chloride concentration, and stream nitrate concentration (Eq. 2).

DOC data was transformed using a negative square root transformation, which inverted the coefficients in the regression equation. When taking into consideration the inversion, the negative total mercury concentration coefficient indicates a positive relationship between total mercury and DOC. This is similar to the relationship between DOC and methyl mercury, where DOC acts as a transport vector of mercury in the stream system. This may be useful for predicting DOC in the streams, but the causative relationship is likely reversed where DOC is a driver of total mercury concentrations in the streams (Grigal 2002). Our regression model also indicates a negative influence of stream nitrate concentrations on DOC. It is possible that nitrate is used up in stream primary productivity thereby increasing stream DOC. This may result in a negative relationship, as nitrate is used up within the stream, DOC is being produced.

Variation in chloride concentration in our streams seems to be influenced by winter road salt at two sites, Mud Lick and Mill Run, indicated by large increases in chloride concentrations in the fall and winter months (Fig. 9). The relationship between chloride and DOC in our streams is coincidental rather than mechanistic. Both are dissolved in the stream water and fluctuate closely with streamflow. The regression model indicates a positive influence of chloride concentration on DOC but there does not appear to be an obvious environmental mechanism to explain this relationship. Studies on the removal of natural organic matter (NOM) and mercury from drinking and surface waters indicate that metal chlorides are effective coagulants and serve to aid in the removal of NOM and DOC from water sources (Matilainen et al. 2010, Henneberry et al. 2011). These studies seem to disagree with our model that suggests a positive influence of stream chloride on DOC concentrations.

CONCLUSION

The life histories of northern dusky, northern two-lined, and Allegheny Mountain dusky salamanders allow them to act as vectors to transport this mercury into the terrestrial environment. Therefore, it is important to identify the factors that influence the accumulation of mercury in these salamanders. Tissue total mercury concentrations are significantly correlated with stream methyl mercury concentrations. This indicates that the majority of mercury that these salamanders are incorporating into their tissues comes from the streams. Regression analysis of stream methyl mercury concentrations and the other water chemistry factors indicates that stream DOC, sulfate, and total mercury concentrations are significant drivers of stream methyl mercury, and therefore salamander tissue total mercury. Stream DOC was also significantly correlated with tissue total mercury in northern dusky and Allegheny Mountain dusky salamanders. Regression analysis of DOC indicates stream chloride and nitrate concentrations to be significantly related to stream DOC.

The correlation analyses used to identify key water chemistry factors correlated with salamander tissue total mercury concentrations are limited in application due to the small sample size of 9 averaged values for each parameter. Calculating the means for each parameter at each site was necessitated by the different temporal scales of sampling for salamanders and water chemistry in this study. In future studies, simultaneous sampling of salamanders and stream water chemistry may improve the statistical power of the data. Having concurrent measurements will allow for more powerful correlation and regression analyses of tissue total mercury and stream water factors. Non-lethal tissue sampling as will also facilitate these comparisons by allowing tissue samples to be taken without damage to the salamander population, which may occur because of the frequent samplings (Bergeron et al. 2010). Longer time-series data of two or more years will also elucidate potential patterns among salamander tissue total mercury concentrations and water chemistry factors.

TABLES AND FIGURES

Table 17 Location of streams surveyed in Garrett County, Maryland, 2010 and number of salamanders collected at each stream.

Stream Name	Latitude	Longitude	Number of Salamanders		
			ND	**NTL**	**MD**
Bear Creek	39.6503	-79.2903	0	2	20
Bear Pen	39.5626	-79.1117	25	24	20
Laurel Run (LR)	39.4882	-79.1531	0	0	20
Little Savage River	39.6169	-79.0249	20	10	20
Mud Lick (ML)	39.6461	-79.0257	30	30	6
Monroe Run (MON)	39.5553	-79.2166	30	16	21
Mill Run (MR)	39.7135	-79.3781	29	20	20
Murley Run (MUR)	39.4878	-79.4612	0	0	19
Poplar Lick (PL)	39.6385	-79.1175	30	11	20
Total			164	113	166

Table 18 Watershed composition and land use of streams sampled in Garrett County, MD. Land use data for our Bear Creek site is not currently available though the watershed is mostly deciduous forest.

	Land Use (%)					Forest type (%)			Total Area
	Wet.	**Ag.**	**Dev.**	**For.**	**Bare**	**Decid.**	**Ever.**	**Mixed**	**(Ha)**
Bear Creek	0	0.82	2.43	96.59	0.17	80.13	16.46	0	95
Bear Pen	0	9.88	1.57	88.07	0.48	84.34	2.44	1.29	867
Laurel Run	0	0.36	11.66	85.26	2.73	84.55	0.7	0	159
L. Sav. River	0.14	0.3	1.95	95.21	0.21	70.96	22.47	1.77	555
Mud Lick	0	29.1	7.73	61.92	1.2	42.92	18.07	0.93	1432
Monroe Run	0	2.68	4.8	92.46	0.05	87.69	3.97	0.8	1201
Mill Run	0	15.61	9.42	74.28	0.59	69.72	3.86	0.7	4365
Murley Run	0	0.01	1.66	98.34	0	90.4	3.55	4.38	290
Poplar Lick	0.18	5.46	3.36	89.74	0.9	67.68	19.81	2.26	2139

Table 19 Methods used in water chemistry analysis of streams in Garrett County, Maryland, 2010.

	Procedure for Sample Preparation	Method of Analysis
Dissolved Organic Carbon	EPA Method 415.3	Ultraviolet Absorbance at 254 nm
Total Suspended Solids	ESS Method 340.3	Filtration and Drying to 103-105 C
Major Anions (Cl^-, NO_3^-, SO_4^{2-})	EPA Method 300 Rev 2.1	Ion Chromatography
Acid Neutralizing Capacity		Gran Analysis Technique
pH	EPA Method 150.1	Electrometric Determination
Total Mercury	EPA Method 1631	Cold Vapor Atomic Fluorescence Spectrophotometry (CVAFS)
Methyl Mercury	EPA Method 1630	Ethylation and Preconcentration Purge and Trap Techniques

Table 20 Mean total mercury concentrations (ng/g), SVL, weight and length for adults salamanders in the 9 study watersheds. All species were not found in all watersheds.

Species	Site	Hg ng/gww	SVL mm	Weight g	Length mm	n
MD	BC	14.1	24	0.34	46	20
	BP	15.7	36	0.90	74	20
	LR	18.4	33	0.79	67	20
	LSR	32.7	35	0.89	72	20
	ML	44.2	37	1.02	77	6
	MON	23.4	37	1.06	75	21
	MR	15.8	34	0.94	72	20
	MUR	24.9	36	0.95	69	19
	PL	20.1	37	1.15	80	20
ND	BP	14.6	45	2.22	86	25
	LSR	35.6	44	2.14	82	21
	ML	28.7	44	2.04	88	30
	MON	16.3	50	3.17	94	30
	MR	17.1	45	2.02	84	29
	PL	19.6	45	2.23	88	30
NTL	BC	27.7	40	0.99	82	2
	BP	24.5	31	0.52	61	24
	LSR	36.5	36	0.80	75	10
	ML	43.4	36	0.78	75	31
	MON	22.9	38	1.03	81	16
	MR	19.4	36	0.84	79	20
	PL	31.4	36	0.93	79	11

Table 21 Pearson product-moment correlation coefficients for ¼ root transformed total mercury concentrations in adult northern dusky (ND) Allegheny Mountain dusky (MD) and northern two-lined (NTL) salamanders and stream water chemistry factors. Asterisks indicate significant correlations (*P* < 0.05). Correlation analyses were run using only data from the sites where the species were found.

	RootTotal Hg ND	**RootTotal Hg NTL**	**RootTotal Hg MD**
pH	-0.708	-0.549	-0.217
ANC	-0.410	-0.296	-0.165
THg	0.843*	0.645	0.542
MeHg	0.888*	0.731	0.699*
DOC	0.940*	0.662	0.726*
TSS	-0.378	-0.193	-0.054
Cl	0.198	-0.010	0.195
NO3	-0.715	-0.585	-0.251
SO4	-0.622	-0.865*	-0.514

Table 22 Mean water chemistry factors for streams sampled in Garrett County, MD (±SE).

	Bear Creek	Bear Pen	Laurel Run	Little Savage	Mill Run	Monroe Run	Mud Lick	Murley Run	Poplar Lick
Mean pH	6.64 ± 0.11	6.49 ± 0.11	6.89 ± 0.36	5.98 ± 0.10	6.70 ± 0.12	6.55 ± 0.11	6.52 ± 0.10	4.59 ± 0.04	6.49 ± 0.10
Mean ANC (μeq/L)	348.7 ± 65.3	182.7 ± 23.3	706.2 ± 254.5	43.9 ± 12.3	471.1 ± 61.7	360.9 ± 50.0	467.8 ± 91.5	-22.3 ± 1.3	293.4 ± 38.3
Mean THg (ng/L)	0.78 ± 0.12	0.66 ± 0.05	1.06 ± 0.26	2.25 ± 0.21	0.52 ± 0.06	0.60 ± 0.06	1.00 ± 0.10	1.47 ± 0.58	0.76 ± 0.09
Mean MeHg (ng/L)	0.07 ± 0.01	0.09 ± 0.02	0.03 ± 0.00	0.21 ± 0.05	0.06 ± 0.01	0.09 ± 0.02	0.15 ± 0.03	0.18 ± 0.05	0.08 ± 0.02
Mean % MeHg	10.10 ± 6.4	12.59 ± 7.7	3.20 ± 2.4	9.43 ± 6.2	12.26 ± 7.2	14.71 ± 6.1	14.56 ± 6.3	14.82 ± 8.9	10.74 ± 6.6
Mean Cl^{-1} (mg/L)	4.40 ± 1.21	1.16 ± 0.09	35.22 ± 4.45	3.10 ± 0.90	90.43 ± 19.1	16.86 ± 1.46	74.78 ± 20.1	0.56 ± 0.03	2.20 ± 0.32
Mean NO_3^{-1} (mg/L)	0.50 ± 0.02	0.50 ± 0.07	0.20 ± 0.02	0.18 ± 0.04	0.55 ± 0.11	0.43 ± 0.09	0.48 ± 0.13	0.05 ± 0.01	0.36 ± 0.06
Mean SO_4^{-2} (mg/L)	9.56 ± 0.28	11.66 ± 0.21	17.06 ± 1.43	8.05 ± 0.78	18.44 ± 1.64	8.13 ± 0.70	8.93 ± 1.26	8.63 ± 0.58	8.02 ±0.44
Mean TSS (mg/L)	2.17 ± 0.56	2.76 ± 0.68	1.67 ± 0.27	2.42 ± 0.92	1.43 ± 0.25	1.17 ± 0.45	1.81 ± 0.61	1.85 ± 0.54	2.44 ± 0.56
Mean DOC (mg/L)	0.51 ± 0.04	0.84 ± 0.06	1.32 ± 0.15	3.51 ± 0.24	1.02 ± 0.07	0.81 ± 0.06	2.06 ± 0.25	1.34 ± 0.20	0.95 ± 0.06

Figure 6 Stream Sites sampled in the Savage River and Youghiogheny River Watersheds, in Garrett County, Maryland.

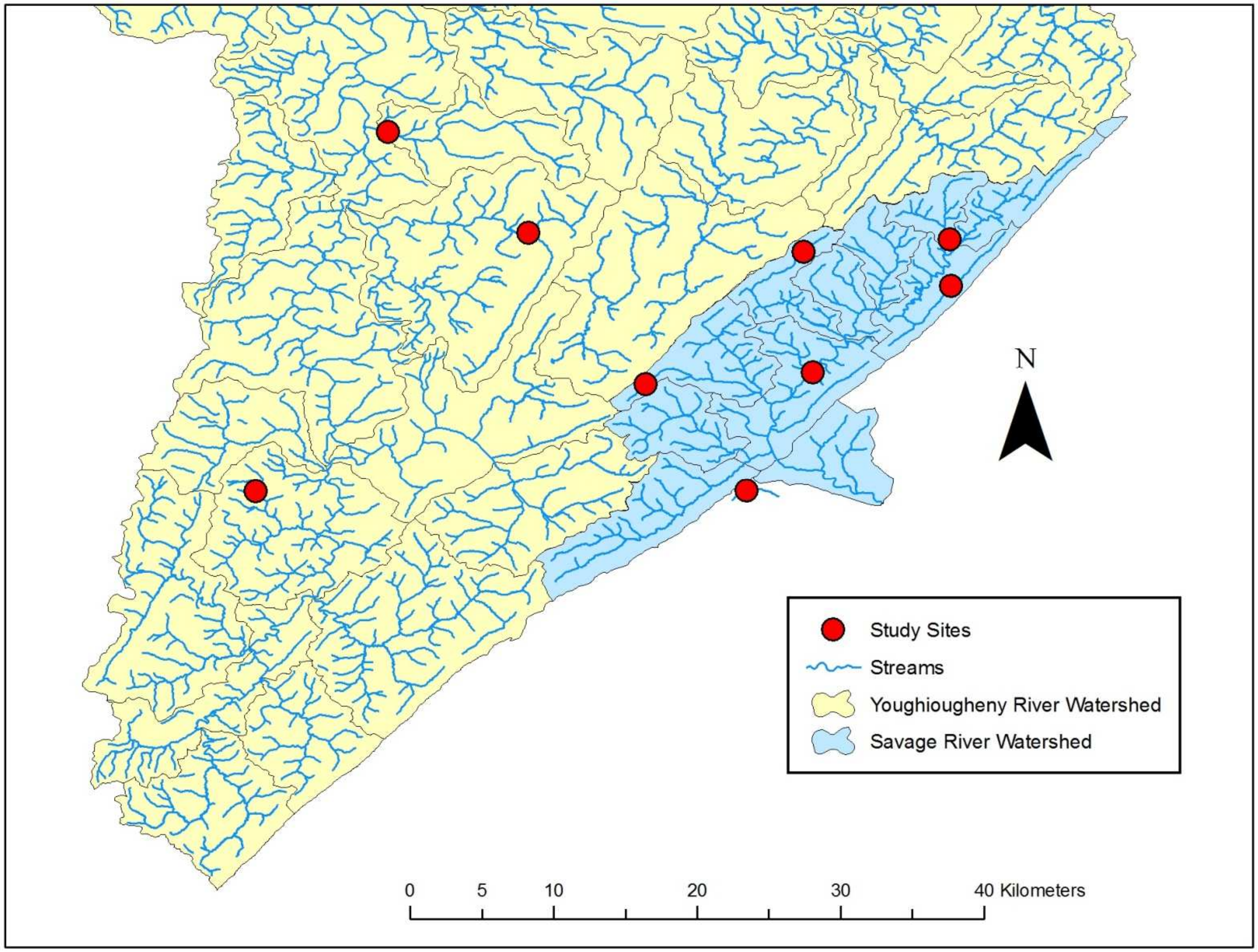


Figure 7 Time series plot of methyl mercury concentrations and sulfate concentrations at Murley Run stream site.

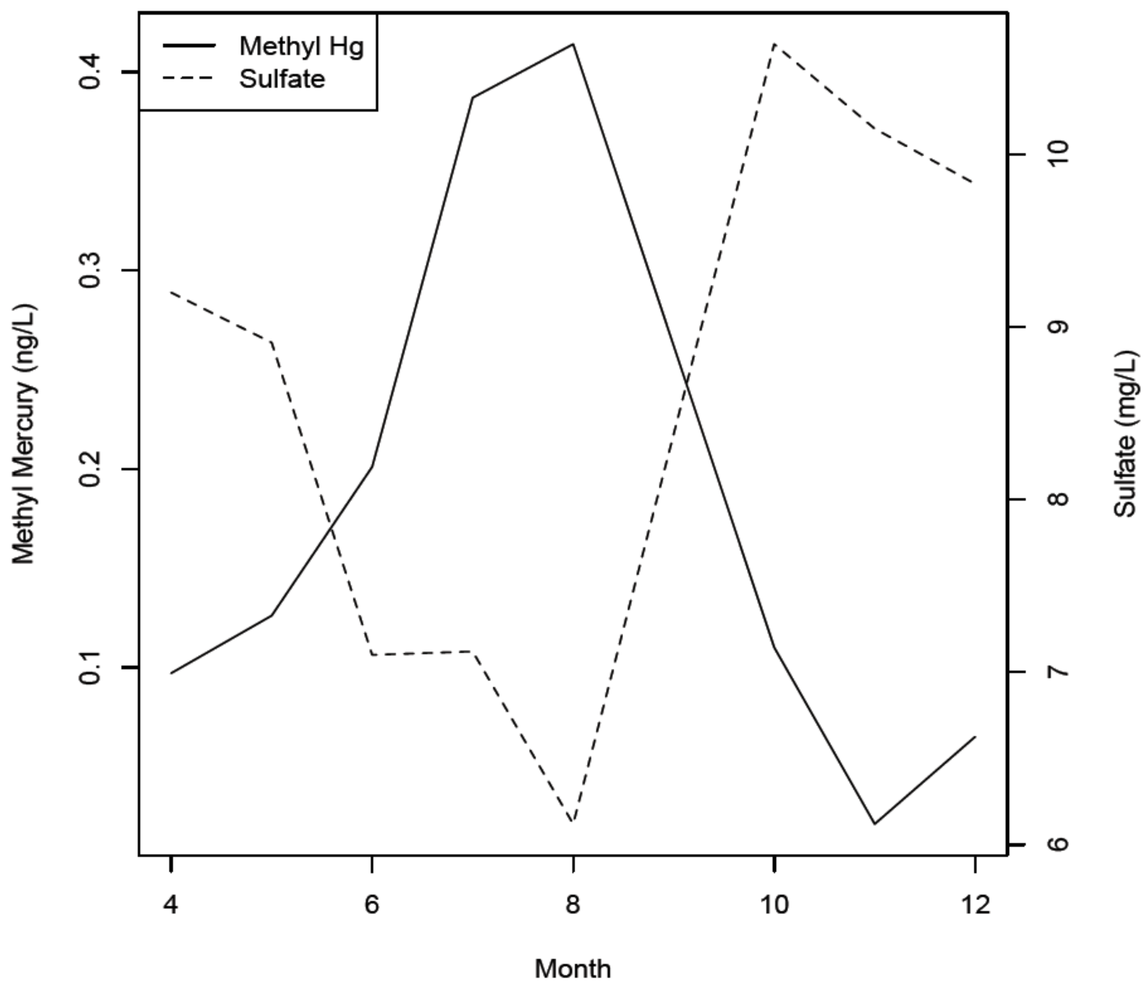


Figure 8 Plot of stream methyl mercury and stream sulfate concentration at Murley Run with regression line.

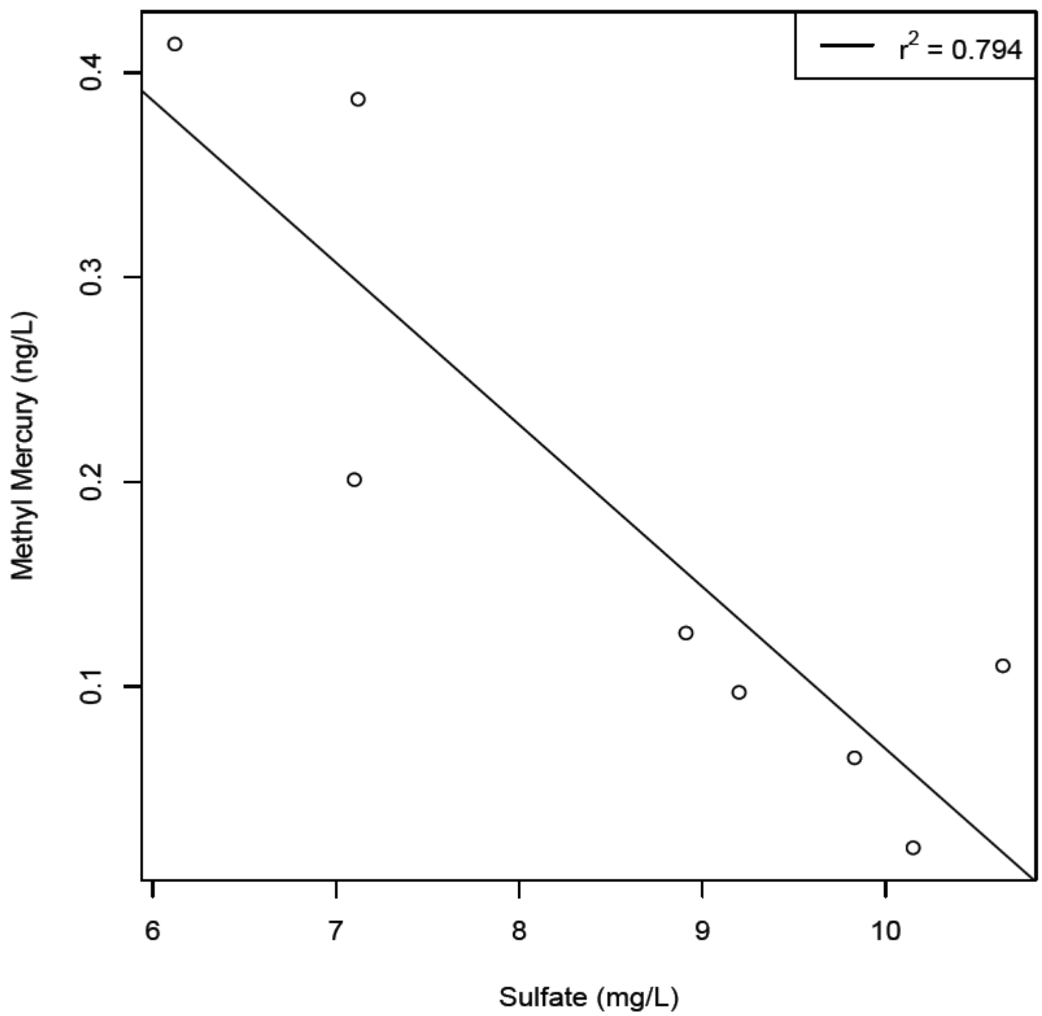


Figure 9 Time series of chloride concentrations at Mill Run and Mud Lick.

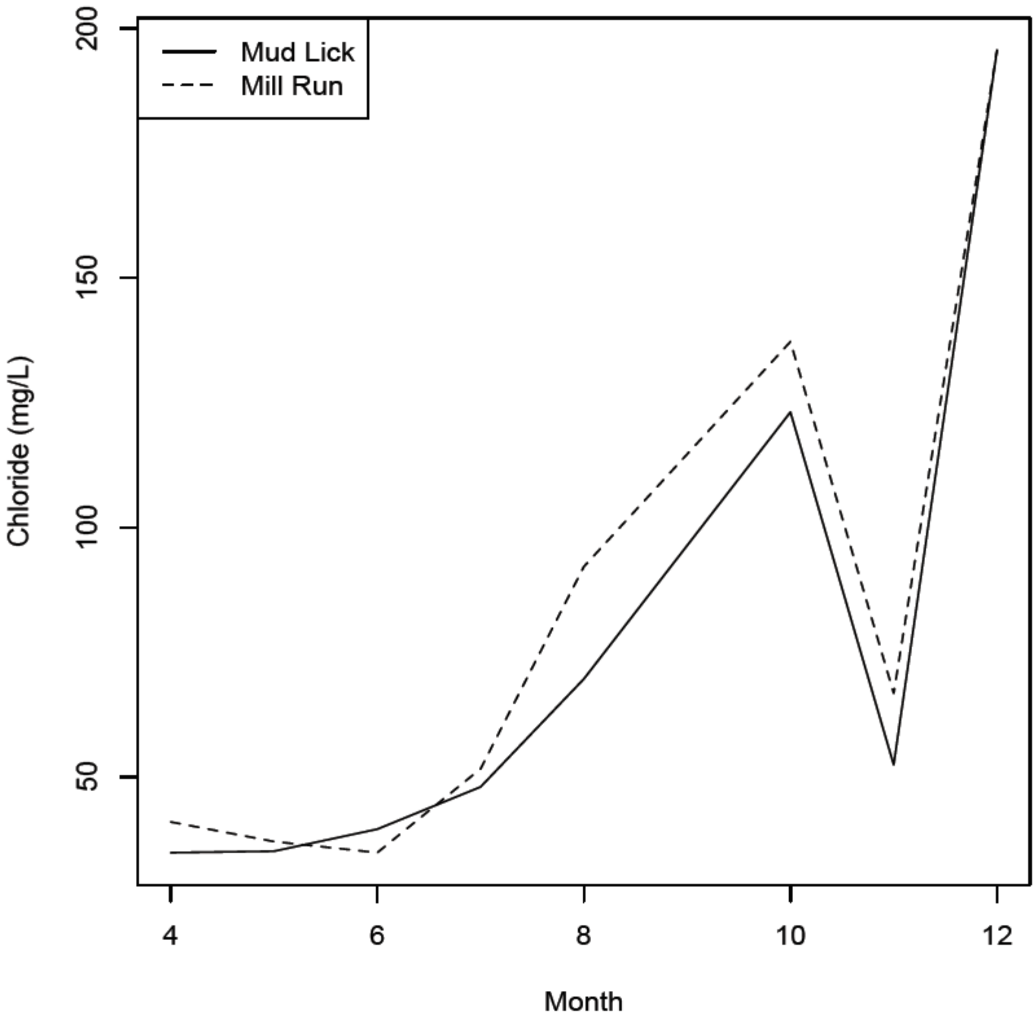
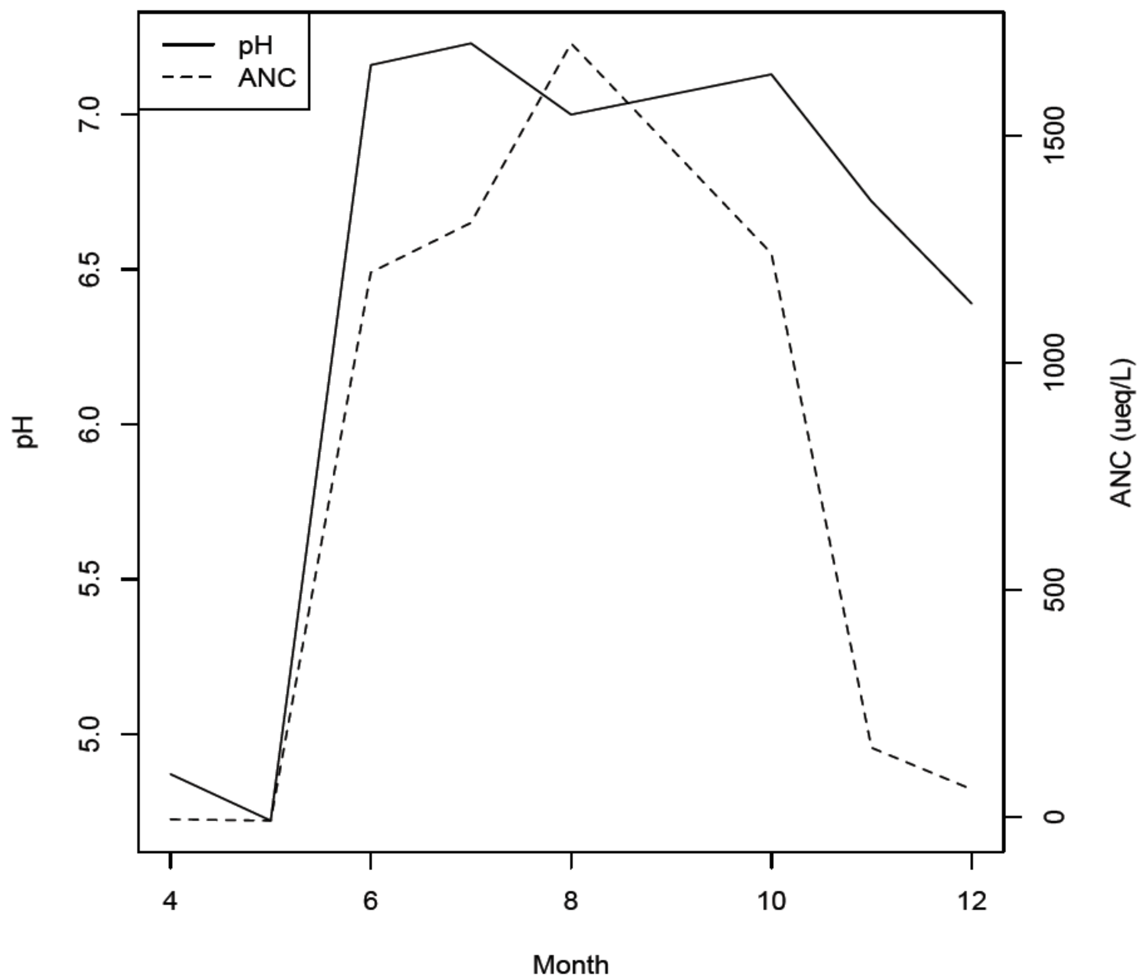


Figure 10 Time series of pH and ANC at Laurel Run in Garrett County, MD in 2010. Remediated with lime added upstream of our sampling site between the May and June samplings, resulting in an increase in both pH and ANC.



LITERATURE CITED

Bank, M.S., Loftin, C., Jung, R. 2005. Mercury Bioaccumulation in Northern Two-Lined Salamanders from Streams in the Northeastern United States. Ecotoxicology 14: 181-191.

Benoit, J.M., Gilmour, C., Heyes, A., Mason, R., Miller, C. 2003. Geochemical and Biological Controls over Methylmercury Production and Degradation in Aquatic Ecosystems. Biogeochemistry of Environmentally Important Trace Elements 19: 262-297.

Bergeron, C.M., Bodinof, C., Unrine, J., Hopkins, W. 2010. Mercury Accumulation Along a Contamination Gradient and Nondestructive Indices of Bioaccumulation in Amphibians. Environmental Toxicology and Chemistry 29, 4: 980-988.

Brigham, M.E., Wentz, D., Aiken, G., Krabbenhoft, D., 2009, Mercury Cycling in Stream Ecosystems. 1. Water Column Chemistry and Transport. Environmental Science and Technology 43, 8: 2720-2725.

Burke, J.N., Bergeron C., Todd, B., Hopkins, W. 2010. Effects of mercury on behavior and performance of northern two-lined salamanders (Eurycea bislineata). Environmental Pollution 158: 3546-3551.

Castro, M.S., Hilderbrand, R., Thompson, J., Heft, A., Rivers, S. 2006. Relationships Between Wetlands and Mercury in Brook Trout. Archives of Environmental Contamination and Toxicology 52: 97-103.

Driscoll, C.T., Han, Y., Chen, C., Evers, D., Lambert, K., Holsen, T., Kamman, N., Munson, R. 2007. Mercury Contamination in Forest and Freshwater Ecosystems in the Northeastern United States. Bioscience 57, 1: 17-28.

Wu, F., Cai, Y., Evans, D., Dillon, P. 2004. Complexation between Hg(II) and Dissolved Organic Matter in Stream Waters: An Apllication of Fluorescence Spectroscopy. Biogeochemistry. 71, 3: 339-351.

Gilmour, C.G., Henry, E.A. (1991). Mercury Methylation in Aquatic Systems Affected by Acid Deposition. Environmental Pollution 71: 131–69.

Gilmour, C.G., Henry, E., Mitchell, R. 1992. Sulfate Stimulation of Mercury Methylation in Freshwater Sediments. Environmental Science and Technology 26: 2281-2287.

Gilmour, C.G., Riedel, G.S. 2000. A Survey of Size Specific Mercury Concentrations in Game Fish from Maryland Fresh and Estuarine Waters. Archives of Environmental Contamination and Toxicology 39: 53-59.

Grigal, D.F. 2002. Inputs and Outputs of Mercury from Terrestrial Watersheds: a Review. Environmental Review 10: 1-39.

Henneberry, Y.K., Kraus, T., Fleck, J., Krabbenhoft, D., Bachand, P., Horwath, W. 2011. Removal of Inorganic Mercury and Methyl Mercury from Surface Waters Following Coagulation of Dissolved Organic Matter with Methal-Based Salts. Science of the Total Environment. 409, 3: 631-637.

Hogan, L.S., Marschall, E., Folt, C., Stein, R. 2007. How Non-native Species in Lake Erie Influence Trophic Transfer of Mercury and Lead to Top Predators. Journal of Great Lakes Research 33, 1: 46-61.

Langley, D. G. 1973. Mercury Methylation in an Aquatic Environment. Water Pollution Control Federation 45, 1: 44-51.

Lanoo, M. 2005. Amphibian Declines: The Conservation Status of United States Species. Berkeley and Los Angeles, CA: University of California Press.

Lindberg, S., Bullock, R., Ebinghaus, R., Engstrom, D., Feng, X., Fitzgerald, W., Pirrone, N., Prestbo, E., Seigneur, C. 2007. A Synthesis of Progress and Uncertainties in Attributing the Sources of Mercury in Deposition. Ambio 37, 1: 19-31.

Mason, R.P., Reinfelder, J., Morel, F. 1996. Uptake, toxicity, and trophic transfer of mercury in a coastal diatom. Environmental Science and Technology 30, 6:1835–1845.

Matilainen, A., Vepsalainen, M., Sillanpaa, M. 2010. Natural Organic Matter Removal by Coagulation during Drinking Water Treatment: A Review. Advances in Colloid and Interface Science. 159, 2: 189-197.

Miller, C.L., Mason, R., Gilmour, C., Heyes, A. 2007. Influence of Dissolved Organic Matter on the Complexation of Mercury Under Sulfidic Conditions. Environmental Toxicology and Chemistry. 26, 4: 624-633.

Petranka, JW. 1998. Salamanders of the United States and Canada. Smithsonian Institution Press, Washington, DC.

Swanson, H.K., Johnston, T., Leggett, W., Bodaly, R., Doucett R., Cunjak, R. 2003. Trophic Positions and Mercury Bioaccumulation in Rainbow Smelt (*Osmerus mordax*) and Native Forage Fishes in Northwestern Ontario Lakes. Ecosystems 6: 289-299.

Shin, E.B., Krenkel, P. 1976. Mercury Uptake by Fish and Biomethylation Mechanisms. Water Pollution Control Federation 48, 3: 473-501.

Ward, D. M., Nislow, K., Folt, C. 2010. Bioaccumulation Syndrome: Identifying Factors that make some Stream Food Webs Prone to Elevated Mercury Bioaccumulation. Annals of the New York Academy of Sciences 1195: 62-83.

CONCLUSIONS

The toxicity of mercury and its prevalence throughout the globe has led to new research into areas of the mercury biogeochemical cycle that have received little attention in the past. Thorough research into the concentrations that can be expected in species of game fish have increased our knowledge of human exposure to mercury, but there are still large gaps in our knowledge of how mercury moves through the biota in the landscape. My research into mercury concentrations in stream salamanders attempted to increase our knowledge of how much mercury is present in biota besides fish. The connection between aquatic and riparian landscapes created by salamanders and other amphibians make them an important area to study mercury concentrations. They are important to help of understand the potential migration of mercury into the terrestrial landscape. I attempted to determine how mercury concentrations change throughout the life cycle of stream salamanders, how mercury concentrations differ between species of salamander with different life history strategies, and how water quality may impact these concentrations.

In five streams mercury concentrations were found to be significantly higher in adult northern two-lined salamanders than in larvae, but at two streams adults and larvae were found to have statistically similar mercury concentrations. I had hypothesized that due to the terrestrial nature of adult northern two-lined diets that larval salamanders would actually have higher total mercury concentrations than adults. This hypothesis assumed that the terrestrial diet of the adults was lower in mercury than the aquatic diet of the larvae. These results may indicate that the terrestrial diets of the adults are higher in mercury than the diets of the larvae. This opens new questions and new research opportunities to truly understand the mercury dynamics in this species of salamander. Are terrestrial macroinvertebrates at these five streams truly higher in mercury, or the adults still consuming mostly aquatic macroinvertebrates? Answering these questions will help us to better how mercury is moving through these ecosystems.

My data showed that adult northern two-lined salamanders had significantly higher mercury concentrations than both adult northern dusky and adult Allegheny Mountain dusky salamanders. I had hypothesized that this would be the case due to the longer aquatic larval stage of the northern two-lined salamanders (1-3 years) compared to the other two species (9-12 months for northern dusky salamanders, and 0-3 months for Allegheny Mountain dusky salamanders). I had also hypothesized that allehgeny mountain dusky salamanders would have the lowest concentrations of mercury due to their more terrestrial life history strategy. Similarly to the comparison of adult to larval northern two-lined salamanders, my data showed Allegheny Mountain dusky salamanders to not have significantly lower mercury concentrations than northern dusky salamanders, despite the assumption of a more terrestrial life strategy and diet. In fact, at two streams, Mud Lick and Monroe Run, Allegheny Mountain dusky salamanders had similar mean mercury concentrations to northern two-lined salamanders. These results, and recent studies into the concentrations of mercury in terrestrial arthropods lend evidence to the conclusion that the terrestrial macroinvertebrates preyed upon by adult northern two-lined salamanders and adult Allegheny Mountain dusky salamanders may not have lower mercury than the aquatic macroinvertebrates consumed by larval salamanders. If these salamanders are in fact consuming mostly terrestrial macroinvertebrates that are lower in mercury, then these adult salamanders should have lower concentrations than larvae and other species that are consuming more aquatic macroinvertebrates.

All of my collected salamanders had mean total mercury concentrations that were significantly positively correlated with stream methyl mercury concentrations. This indicates that much of the mercury accumulated by these salamanders originates in the streams. This may explain the unexpectedly high concentrations in adult northern two-lined and Allegheny Mountain dusky salamanders. This tissue mercury concentration correlation with stream methyl mercury concentrations could indicate more aquatic diets of these salamanders than were assumed at the outset of the project. My data indicates that stream methyl mercury concentrations are significantly affected by stream total mercury concentrations, stream DOC concentrations, and stream sulfate concentrations. These regression results imply that much of the methyl mercury present in the streams is being produced locally, rather than being transported long distances.

Total mercury concentrations in Allegheny Mountain dusky and northern dusky salamanders were also significantly positively correlated with DOC. The role of DOC as a transport vector for both inorganic and methyl mercury may explain this result. My data suggests a significant link between DOC and stream total mercury concentrations, stream chloride concentrations, and stream nitrate concentrations. The transport vector link between mercury and DOC explain part of these results. The link between chloride, nitrate, and DOC may solely be a coincidence related to all three existing in the streams in the dissolved form.

The results of my project indicate that much of the variation in total mercury concentrations in stream salamanders may be attributable to dietary variation within and across populations. To truly understand the dynamics of mercury in salamanders we need to understand the role of dietary sources of mercury in these organisms. A better understanding of the role diet plays in the accumulation of mercury in salamanders will also help us to better understand the role played by other factors such as life history strategy and stream water quality. Future studies should attempt to identify the dietary composition in local populations of stream salamander. Once the composition of the salamander diets has been identified, the variations in mercury concentrations in those prey items should be researched to identify the mercury concentrations being contributed to salamanders from these sources.

APPENDIX A: TOTAL MERCURY CONCENTRATIONS IN SEAL SALAMANDERS AND EASTERN RED-SPOTTED NEWTS COLLECTED FROM STREAMS IN GARRETT COUNTY, MD.

Seal Salamanders and eastern red-spotted newts were not widespread enough to allow inclusion in statistical analyses in the main thesis. Total mercury concentrations were measured for these species (Table 23). Weight, SVL, and total length were also measured for each individual salamander (Table 24).

The overall mean tissue total mercury concentration for seal salamanders was 18.72 ± 1.97 ng g^{-1} within a range of 6.26 ng g^{-1} to 79.42 ng g^{-1}. Across individual streams mean tissue total mercury ranged from 11.37 ± 0.69 ng g^{-1} at Bear Pen to 39.94 ± 6.49 ng g^{-1} at Little Savage River.

The overall mean tissue total mercury concentration for adult eastern red-spotted newts was 28.27 ± 1.53 ng g^{-1} within a range of 5.84 ng g^{-1} to 59.97 ng g^{-1}. across individual streams mean tissue total mercury concentrations ranged from 20.68 ± 2.11 ng g^{-1} at Mill Run to 29.91 ± 2.38 ng g^{-1} at Mud Lick. The overall mean tissue total mercury concentration for eft eastern red-spotted newts was 14.37 ± 4.92 ng g^{-1} within a range of 7.48 ng g^{-1} to 33.49 ng g^{-1}. Across individual streams mean tissue total mercury concentrations were 8.11 ± 0.49 ng g^{-1} at Mill Run and 23.76 ± 9.73 ng g^{-1} at Mud Lick.

Table 23 Mean total mercury concentrations (±SE) for seal salamanders and eastern red-spotted newts collected from streams in Garrett County, MD.

	Mean THg (ng g^{-1})					
Site	**Seal Salamanders**		**Eastern Red-Spotted Newt**			
	Adult	**n**	**Adult**	**n**	**Eft**	**n**
BC	13.88 ± 2.36	21				
BP	11.37 ± 0.69	11				
LR	21.13 ± 3.60	9				
LSR	39.94 ± 6.49	11				
ML			29.91 ± 2.38	32	23.76 ± 9.73	2
MON	11.48 ± 0.80	10				
MR			20.68 ± 2.11	2	8.11 ± 0.49	3
PL			26.98 ± 2.02	29		
Mean	18.72 ± 1.97	62	28.27 ± 1.53	63	14.37 ± 4.92	5

Table 24 Mean weight (±SE), SVL (±SE), and total length (±SE) for seal salamander (S) and eastern red-spotted newts (RSN) sampled from Garrett County, MD.

	Mean Weight (g)			Mean SVL (mm)			Mean Length (mm)		
	S	RSN		S	RSN		S	RSN	
Site	**Adult**	**Adult**	**Eft**	**Adult**	**Adult**	**Eft**	**Adult**	**Adult**	**Eft**
BC	1.68 ± 0.46			35.51 ± 3.25			63.67 ± 5.65		
BP	1.50 ± 0.34			38.84 ± 3.08			81.43 ± 5.87		
LR	3.30 ± 0.71			48.30 ± 3.80			98.01 ± 6.12		
LSR	4.06 ± 0.81			51.16 ± 4.41			99.63 ± 8.99		
ML		3.54 ± 0.11	2.70 ± 0.36		47.01 ± 0.46	36.05 ± 10.15		95.06 ± 1.05	85.95 ± 4.05
MON	3.30 ± 0.78			47.69 ± 4.78			94.14 ± 8.99		
MR		3.58 ± 0.45	2.24 ± 0.28		44.00 ± 2.90	41.40 ± 1.80		87.00 ± 7.60	82.83 ± 4.03
PL		3.26 ± 0.10			45.40 ± 0.56			93.51 ± 1.15	

APPENDIX B: TISSUES METHYL MERCURY ANALYSIS OF SELECTED SALAMANDERS

A small subset of salamanders (26) was analyzed for methyl and total mercury to ascertain the percent of total mercury present in tissues as methyl mercury (Table 25, 26). The methyl mercury analysis did not meet QA/QC standards and therefore this data was not included in the main thesis. The methyl mercury concentrations measured in the DORM-3 tissue samples used as independent checks of the digestion and analysis procedures were not within the allowable range (Table 27).

The mean percent of total mercury present as methyl mercury for northern two-lined salamanders was 70.58 % within a range of 54.80 % to 86.09 %. This is lower than the percent methyl mercury range of 73 % to 97 % reported by Bank et al. (2005), though it is similar to the percent methyl mercury of 61.2% reported by Bergeron et al. (2010).

The mean percent methyl mercury for northern dusky salamanders was 72.06 % within a range of 51.30 % to 85.14 %. The mean percent methyl mercury for Allegheny Mountain dusky salamanders was 67.34 % within a range of 55.04 % to 81.77 %.

Table 25 Methyl mercury concentrations, total mercury concentrations, and the percent methyl mercury for a subset of salamanders collected from streams in Garrett County, MD.

		Mercury		
Sample	**Species**	**Methyl**	**Total**	**Percent Methyl**
		ng g^{-1}	**ng g^{-1}**	**%**
FBP-NTLA-11	NTL	15.52	21.55	72.03
FLSR-NTLA-11	NTL	21.82	26.25	83.15
FLSR-NTLA-16	NTL	27.02	37.14	72.75
FLSR-NTLA-17	NTL	16.27	29.69	54.80
FLSR-NTLA-18	NTL	20.82	36.47	57.10
FLSR-NTLA-19	NTL	21.33	34.40	62.00
FLSR-NTLA-21	NTL	15.83	22.96	68.93
FLSR-NTLA-22	NTL	14.15	18.06	78.34
FLSR-NTLA-23	NTL	37.30	43.33	86.09
FMON-NDA-15	ND	9.16	13.12	69.85
FPL-NDA-11	ND	15.88	18.65	85.14
FPL-NDA-12	ND	22.57	30.91	73.00
FPL-NDA-14	ND	18.05	31.56	57.20
FPL-NDA-15	ND	12.95	16.31	79.36
FPL-NDA-16	ND	21.11	27.11	77.87
FPL-NDA-17	ND	12.81	18.05	70.93
FPL-NDA-18	ND	13.19	15.73	83.85
FPL-NDA-19	ND	6.46	12.58	51.30
FBP-MDA-11	MD	11.16	16.41	68.00
FLSR-MDA-11	MD	16.96	26.52	63.93
FMON-MDA-2	MD	14.20	22.04	64.44
FMON-MDA-3	MD	9.60	17.45	55.04
FMON-MDA-4	MD	18.55	23.63	78.51
FMON-MDA-7	MD	17.87	21.85	81.77
FMR-MDA-9	MD	7.88	12.18	64.66
FPL-MDA-11	MD	15.90	25.48	62.40
Mean		16.71	23.82	70.09

Table 26 Mean methyl mercury concentrations, mean total mercury concentrations, and mean percent methyl mercury for northern two-lined salamanders (NTL), northern dusky salamanders (ND), and Allegheny Mountain dusky salamanders (MD).

Species	**Mean Methyl ng g^{-1}**	**Mean Total ng g^{-1}**	**Mean Percent Methyl %**
MD	14.01	20.69	67.34
ND	14.69	20.45	72.06
NTL	21.12	29.98	70.58

APPENDIX C: SALAMANDER ENCOUNTER DATA INCLUDING SHANNON DIVERSITY INDEX FOR INDIVIDUAL STREAMS IN GARRETT COUNTY, MD.

Table 27 Count of salamander species and individuals encountered during spring sampling and Shannon Diversity Index values for individual streams in Garrett County, MD.

			Spring						
	BC	**BP**	**LR**	**LSR**	**MR**	**MON**	**ML**	**MUR**	**PL**
Northern Dusky	0	15	0	12	9	10	12	0	11
Northern Two-Lined	0	35	0	0	10	11	30	0	13
Eastern Red-Spotted Newt	0	0	0	0	2	0	13	0	15
Mountain Dusky	12	11	8	8	16	8	14	11	7
Seal Salamander	5	6	6	5	1	7	0	0	0
Spring Salamander	0	2	0	1	0	0	0	0	0
Red Salamander	0	0	0	0	0	0	0	0	0
Long-Tailed	0	0	0	0	0	0	0	0	0
Total Species	2	5	2	4	5	4	4	1	4
Total Individuals	17	69	14	26	38	36	69	11	46
Shannon Diversity Index	0.606	1.284	0.683	1.162	1.307	1.371	1.304	0.000	1.351
Man Hours	3	5	3	5	6	4	8	3	5

Table 28 Count of salamander species and individuals encountered during summer sampling and Shannon Diversity Index values for individual streams in Garrett County, MD.

	Summer								
	BC	**BP**	**LR**	**LSR**	**MR**	**MON**	**ML**	**MUR**	**PL**
Northern Dusky	0	14	0	11	14	13	16	0	11
Northern Two-Lined	10	13	0	0	31	14	27	0	12
Eastern Red-Spotted Newt	0	0	0	0	3	0	15	0	11
Mountain Dusky	15	14	12	13	17	12	16	13	13
Seal Salamander	11	13	10	15	0	12	0	0	0
Spring Salamander	0	0	0	2	0	0	0	0	0
Red Salamander	0	0	0	0	1	0	0	0	0
Long-Tailed	0	0	0	0	1	0	0	0	0
Total Species	3	4	2	4	6	4	4	1	4
Total Individuals	36	54	22	41	67	51	74	13	47
Shannon diversity Index	1.083	1.386	0.689	1.232	1.296	1.384	1.354	0.000	1.384
Man Hours	5	8	4	6	10	8	10	4	8

Table 29 Count of salamander species and individuals encountered during fall sampling and Shannon Diversity Index values for individual streams in Garrett County, MD.

	Fall								
	BC	**BP**	**LR**	**LSR**	**MR**	**MON**	**ML**	**MUR**	**PL**
Northern Dusky	0	5	0	2	21	19	14	0	22
Northern Two-Lined	13	12	0	30	24	13	26	0	20
Eastern Red-Spotted Newt	0	0	0	0	0	0	16	0	11
Mountain Dusky	22	12	11	13	15	24	6	8	13
Seal Salamander	12	9	9	4	0	16	0	0	0
Spring Salamander	0	1	0	0	0	0	0	0	0
Red Salamander	0	0	0	0	0	0	0	0	0
Long-Tailed	0	0	0	0	0	0	0	0	0
Total Species	3	5	2	4	3	4	4	1	4
Total Individuals	47	39	20	49	60	72	62	8	66
Shannon diversity Index	1.059	1.421	0.688	0.987	1.081	1.361	1.276	0.000	1.347
Man Hours	6	8	4	8	6	8	10	3	8

LITERATURE CITED

Balogh SJ, Yabing H, Offerman H, Meyer M, Johnson D. Methylmercury in rivers draining cultivated watersheds. Sci Total Environ 2003; 304: 305-313.

Bank MS, Loftin C, Jung R. Mercury Bioaccumulation in Northern Two-Lined Salamanders from Streams in the Northeastern United States. Ecotoxicology 2005; 14: 181-191.

Ben-Ari E. A New Piece in the Puzzle of Global Amphibian Declines. Bioscience 2005; 55, 1: 96.

Benoit, J.M., Gilmour, C., Heyes, A., Mason, R., Miller, C. Geochemical and Biological Controls over Methylmercury Production and Degradation in Aquatic Ecosystems. Biogeochemistry of Environmentally Important Trace Elements 2003; 19: 262-297.

Bergeron CM, Bodinof C, Unrine J, Hopkins W. Mercury Accumulation Along a Contamination Gradient and Nondestructive Indices of Bioaccumulation in Amphibians. Environ Toxicol Chem 2010; 29, 4: 980-988.

Boone MD, James S. Interactions of an Insecticide, Herbicide, and Natural Stressors in Amphibian Community Mesocosms. Ecol Appl 2003; 13, 3: 829-841.

Brigham ME, Wentz D, Aiken G, Krabbenhoft D. Mercury Cycling in Stream Ecosystems. 1. Water Column Chemistry and Transport. Environ Sci Technol 2009; 43,8: 2720-2725.

Brooks S, Moore C, Lew D, Lefer B, Huey G, Tanner D. Temperature and sunlight controls of mercury oxidation and deposition atop the Greenland ice sheet. Atmos Chem Phys Discus 2011; 11: 3663-3691.

Burke JN, Bergeron C, Todd B, Hopkins W. Effects of mercury on behavior and performance of northern two-lined salamanders (Eurycea bislineata). Environ Pollut 2010; 158: 3546-3551.

Castro MS, Hilderbrand R, Thompson J, Heft A, Rivers S. Relationships Between Wetlands and Mercury in Brook Trout. Arch Environ Contam Toxicol 2007; 52: 97-103.

Castro, MS, Hilderbrand, RH, Kaumeyer M. Mercury Concentrations in Northern Two-Lined Salamanders from Stream Ecosystems in Garrett County, Maryland. Arch Environ Contam and Toxicol 2018; 75: 17-24.

Cremona F, Planas D, Lucotte M. Assessing the importance of macroinvertebrate trophic dead ends in the lower transfer of methylmercury in littoral food webs. Can J Fish Aquat Sci 2008; 65, 9: 2043-2052.

Driscoll CT, Han Y, Chen C, Evers D, Lambert K, Holsen T, Kamman N, Munson R. Mercury Contamination in Forest and Freshwater Ecosystems in the Northeastern United States. Bioscience 2007; 57, 1: 17-28.

Fitzgerald WF, Engstrom DR, Mason RP, Nater EA. The case for atmospheric mercury contamination in remote areas. Environ Sci Technol 1998; 32:1–7.

Fitzpatrick LC, Bristol JR, Stokes RM. Thermal Acclimation and Metabolic Rates in the Dusky Salamander, *Desmognathus fuscus*. Comp Biochem Physiol 1972; 41a: 89-96.

Fitzpatrick LC. Energy Allocation in the Allegheny Mountain Salamander, Desmognathus Ochrophaeus. Ecol Monogr 1973; 43,1: 43-58.

Fitzpatrick LC. Influence of Seasonal Temperatures on the Energy Budget and Metabolic Rates of the Northern Two-Lined Salamander, *Eurycea bislineata bislineata*. Comp Biochem Physiol 1973; 45, 3: 807-818.

Gilmour CG and Henry EA. Mercury Methylation in Aquatic Systems Affected by Acid Deposition. Environ Pollut 1991; 71: 131–69.

Gilmour, C.G., Henry, E., Mitchell, R. Sulfate Stimulation of Mercury Methylation in Freshwater Sediments. Environmental Science and Technology 1992; 26: 2281-2287.

Gilmour, C.G., Riedel, G.S. 2000. A Survey of Size Specific Mercury Concentrations in Game Fish from Maryland Fresh and Estuarine Waters. Archives of Environmental Contamination and Toxicology 39: 53-59.

Grigal, D.F. Inputs and Outputs of Mercury from Terrestrial Watersheds: a Review. Environmental Review 2002; 10: 1-39.

Hatch AC, Blaustein A. Combined Effects of UV-B Radiation and Nitrate Fertilizer on Larval Amphibians. Ecol Appl 2003; 13, 4: 1083-1093.

Henneberry, Y.K., Kraus, T., Fleck, J., Krabbenhoft, D., Bachand, P., Horwath, W. Removal of Inorganic Mercury and Methyl Mercury from Surface Waters Following Coagulation of Dissolved Organic Matter with Methal-Based Salts. Science of the Total Environment 2011; 409, 3: 631-637.

Hogan, L.S., Marschall, E., Folt, C., Stein, R. How Non-native Species in Lake Erie Influence Trophic Transfer of Mercury and Lead to Top Predators. Journal of Great Lakes Research 2007; 33, 1: 46-61.

Kelly EN, Schindler D, St. Louis V, Donald D, Vladicka K. Forest Fire Increases Mercury Accumulation by Fishes via Food Web Restructuring and Increased Mercury Inputs. Proc Nat Acad Sci USA 2006; 103, 51: 19380-19385.

Langley, D. G. Mercury Methylation in an Aquatic Environment. Water Pollution Control Federation 1973; 45, 1: 44-51.

Lanoo M. Amphibian Declines: The Conservation Status of United States Species. Berkeley and Los Angeles, CA: University of California Press; 2005.

Lenkowski JR, Reed J, Deininger L, McLaughlin K. Perturbation of Organogenesis by the Herbicide Atrazine in the Amphibian Xenopus laevis. Environ Health Perspect 2008; 116, 2: 223-230.

Lindberg, S., Bullock, R., Ebinghaus, R., Engstrom, D., Feng, X., Fitzgerald, W., Pirrone, N., Prestbo, E., Seigneur, C. A Synthesis of Progress and Uncertainties in Attributing the Sources of Mercury in Deposition. Ambio 2007; 37, 1: 19-31.

Mason RP, Laporte JM, Andres S. Factors Controlling the Bioaccumulation of Mercury, Methylmercury, Arsenic, Selenium, and cadmium by Freshwater Invertebrates and Fish. Arch Environ Contam Toxicol 2000; 38: 283-297.

Matilainen, A., Vepsalainen, M., Sillanpaa, M. Natural Organic Matter Removal by Coagulation during Drinking Water Treatment: A Review. Advances in Colloid and Interface Science 2010; 159, 2: 189-197.

Mendelson III JR. Confronting Amphibian Declines and Extinctions. Science 2006; 313, 5783: 48.

Miller, C.L., Mason, R., Gilmour, C., Heyes, A. Influence of Dissolved Organic Matter on the Complexation of Mercury Under Sulfidic Conditions. Environmental Toxicology and Chemistry 2007; 26, 4: 624-633.

Miller EK, VanArsdale A, Keeler JG, Chalmers A, Poissant L, Kamman NC, Brulotte R. Estimation and mapping of wet and dry mercury deposition across northeastern North America. Ecotoxicology 2005; 14: 53–70.

Moore CW, Cosby B, Galloway J, Castro M. Relationship Between pH and Stream Water Total Mercury Concentrations in Shenandoah National Park. Water Air Soil Pollut 2009; 201: 233-238.

Moore CW, Castro M, Brooks S (2011) Modified Bowen Ratio Fluxes of Gaseous Elemental Mercury in Western Maryland. Atmos Environ, In Revision.

Morel FM, Kraepiel A, Amyot M. The Chemical Cycle and Bioaccumulation of Mercury. Annu Rev Ecol Syst 1998; 29: 543-566.

National Atmospheric Deposition Program / Mercury Deposition Network (NADP/MDN). (2004). NADP Program Office, Illinois State Water Survey, 2204 Griffith Drive, Champaign, IL 61820, http://nadp.sws.uiuc.edu/.

Peckenham JM, Kahl J, Nelson S, Johnson K, Haines T. Landscape Controls on Mercury in Streamwater at Acadia National Park, USA. Environ Monit Assess 2007; 126: 97-104.

Petranka JW. Ontogeny of the Diet and Feeding Behavior of *Eurycea bislineata* Larvae. J Herpetol 1984; 8, 1: 48-55.

Petranka JW. Salamanders of the United States and Canada. Washington D.C. Smithsonian Institution Press; 1998.

Jose Pinheiro, Douglas Bates, Saikat DebRoy, Deepayan Sarkar and the R Development Core Team (2011). nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1-98.

Prepas EE, Gabos S, Strachan W, Weiping Z. Methyl mercury concentrations in macroinvertebrates and fish from burned and undisturbed lakes on the Boreal Plain. Can J Fish Aquat Sci 2005; 62, 9: 1963-1977.

R Development Core Team (2009). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Rimmer CC, Miller EK, McFarland KP, Taylor RJ, Faccio FD. Mercury bioaccumulation and trophic transfer in the terrestrial food web of a montane forest. Ecotoxicol 2010; 19: 697-709.

Rouse JD, Bishop C, Struger J. Nitrogen Pollution: An Assessment of Its Threat to Amphibian Survival. Environ Health Perspect 1999; 107, 10: 799-803.

Rovito SM, Parra-Olea G, Vasquez-Almazan C, Papenfuss T, Wake D. Dramatic declines in neotropical salamander populations are an important part of the global amphibian crisis. Proc Nat Acad Sci USA 2009; 106: 3231-3236.

Schiesari L, Grillitsch B, Grillitsch H. Biogeographic Biases in Research and Their Consequences for Linking Amphibian Declines to Pollution. Conserv Biol 2007; 21, 2:465-471.

Shanley JB, Kamman N, Clair T, Chalmers A. Physical Controls on Total and Methylmercury Concentrations in Streams and Lakes of the Northeastern USA. Ecotoxicol 2005; 14: 125-134.

Shin, E.B., Krenkel, P. Mercury Uptake by Fish and Biomethylation Mechanisms. Water Pollution Control Federation 1976; 48, 3: 473-501.

Southerland MT, Jung R, Baxter D, Chellman I, Mercurio G, Volstad J. Stream Salamanders as Indicators of Stream Quality in Maryland, USA. Appl Herpetol 2004; 2: 23-46.

Stokstad E. Global Survey Documents Puzzling Decline of Amphibians. Science 2004; 306, 5695: 391.

Storrs SI, Kiesecker J. Survivorship Patterns of Larval Amphibians Exposed to Low Concentrations of Atrazine. Environ Health Perspect 2004; 112, 10:1054-1057.

Swanson, H.K., Johnston, T., Leggett, W., Bodaly, R., Doucett R., Cunjak, R. Trophic Positions and Mercury Bioaccumulation in Rainbow Smelt (*Osmerus mordax*) and Native Forage Fishes in Northwestern Ontario Lakes. Ecosystems 2003; 6: 289-299.

Taylor B, Skelly D, Demarchis L, Slade M, Galusha D, Rabinowitz P. Proximity to Pollution Sources and Risk of Amphibian Limb Malformation. Environ Health Perspect 2005; 113, 11: 1497-1501.

Ward, D. M., Nislow, K., Folt, C. Bioaccumulation Syndrome: Identifying Factors that make some Stream Food Webs Prone to Elevated Mercury Bioaccumulation. Annals of the New York Academy of Sciences 2010; 1195: 62-83.

Williams RN, Bos D, Gopurenko D, DeWoody J. Amphibian Malformations and Inbreeding. Biol Letters 2000; 4: 549-552.

Wolfe MF, Schwarzbach S, Sulaiman RA. Effects of Mercury on Wildlife: A Comprehensive Review. Environ Toxicol Chem 1998; 17: 146–160.

Wu, F., Cai, Y., Evans, D., Dillon, P. Complexation between Hg(II) and Dissolved Organic Matter in Stream Waters: An Application of Fluorescence Spectroscopy. Biogeochemistry 2004; 71, 3: 339-351.

Zizek S, Horvat M, Gibicar D, Fajon V, Toman M. Bioaccumulation of mercury in benthic communities of a river ecosystem affected by mercury mining. Sci Total Environ 2007; 377, 2: 407-415.